2. Literature Review

Viruses have been reported to be responsible for crop losses worldwide for centuries. In India, chronic viral problems have evolved during past few years. Several new and recombinant viral strains with increasing host range are being noted (Schubert *et al.*, 2015; Zhou *et al.*, 2015; Chikh-Ali *et al.*, 2016; Green *et al.*, 2018). The study of the viruses, their symptomology and genome structure are important factors in understanding viral diseases. At the onset of the present study, *Potyvirus* was consistently detected in most of the areas covering the region of the present study. Other viruses that were detected were *Emaravirus* and *Potexvirus.* Hence a selective review of these RNA viruses and their management strategies has been carried out before going ahead with the present work. For convenience, the present review has been grouped into some headings, which are as follows:

- General outline of RNA viruses
- Classification of plant RNA viruses
- Disease symptoms associated with some selected RNA viruses
- Important characteristics of *Potyvirus*, *Potexvirus* and *Emaravirus*
- Insect vectors
- Occurrence of plant diseases caused by selected RNA viruses
- Virus detection methods and disease diagnosis
- Disease management

2.1. General outline of RNA viruses

RNA viruses are the most widely distributed virus family, comprising 70% of all plant viruses and vary remarkably in genome structure. The error rate of the enzymes involved in RNA replication is very high. As a result these viruses usually show much higher mutation rates than do the DNA viruses. Mutation rates of these viruses lead to the continuous generation of virus variants which show great adaptability to new hosts. The viral RNA may be single-stranded (ss) or double-stranded (ds), and the genome may

occupy a single RNA segment or may be segmented into two or more separate segments. The single-stranded genome may be either a sense strand (plus strand), which can function as messenger RNA (mRNA), or an antisense strand (minus strand), which is complementary to the sense strand and cannot function as mRNA in protein translation. Sense viral RNA can directly replicate into the host cells, as it can function as mRNA and initiate translation of virus-encoded proteins. Antisense RNA, on the other hand, has no translational function (Gelderblom, 1996). Studies on viral infection in plant hosts have undoubtedly exemplified the localization and identification of specific viral and host participants at plasmodesmata during viral local cell-to-cell spread from the initially infected cell followed by vascular-mediated spread to distant plant tissues (Kumar *et al.*, 2014).

2.2. Classification of plant RNA viruses

Viruses may be grouped on the basis of size and shape, chemical composition and structure of the genome, and mode of replication. Based on the morphology of nucleocapsid, RNA viruses may be grouped into filamentous, pleomorphic, spherical etc (Gelderblom, 1996). The International Committee on Taxonomy of Viruses (ICTV) began to devise and implement rules for the naming and classification of viruses early in the 1970s, an effort that continues to the present. The ICTV is the only body charged by the International Union of Microbiological Societiy with the task of developing, refining and maintaining universal virus taxonomy (Mayo and Martelli, 1993). Now-a-days, the widely accepted virus classification is Baltimore classification of viruses based on genome structure (Baltimore, 1971). The RNA viruses are classified into four classes: 1. Double stranded RNA virus, 2. Single stranded positive sense RNA virus, 3. Single stranded negative sense RNA virus and 4. Single stranded retro sense RNA virus (Hull, 2002). ICTV in its 10th report has published 71 families of RNA viruses with 304 accepted genera (Website reference 2, https://talk.ictvonline.org/files/master-species-lists/m/msl/6776).

2.3. Disease symptoms associated with some selected RNA viruses

Holmes *et al.* (1948) reported that symptoms may vary with the virus involved, the species of plant infected and the environmental conditions. In some cases certain environmental conditions bring out symptoms while other conditions mask or hide symptoms. Some symptoms associated with virus infections are reduced growth resulting in stunting, mosaic pattern of light and dark green (or yellow and green) on the leaves, malformation of leaves or growing points, yellow streaking of leaves (especially monocots), yellow spotting on leaves, ring spots or line patterns on leaves, cup-shaped leaves, uniform yellowing, bronzing or reddening of foliage, flower color breaking, distinct yellowing only of veins, crinkling or curling of margins of leaves (Kim *et al.*, 2010; Laney *et al.,* 2011; Mansilla *et al.* 2013; Mohammed *et al.* 2012; Mathioudakis *et al.* 2012; Babu *et al.* 2012; Babu *et al.,* 2014; Baker *et al.,* 2014; Di Bello *et al.,* 2015). Some symptoms those were reported to be associated with some selected viral diseases (2001-2018) have been presented in the Table 2.1.

Table 2.1. Disease symptoms associated with some major RNA viral diseases affecting various host plants

Name of the disease	Affected part/Plant	Symptoms	Reference
Oat necrotic mottle virus	Oat	Mosaic symptoms on leaves	Chen *et al.* (2001)
Tomato mild mottle virus	Tomato, *Datura stramonium* and *Solanum nigrum*	Mosaic symptoms on leaves	Monger *et al.* (2001)
Pepino mosaic virus	Tomato	Mosaic, distortion, yellowing of leaves	Mumford and Metcalfe (2001)
Watermelon mosaic virus-2	Cucurbits	Chlorotic mottle and mosaic. Fruits are often stunted and distorted.	Nono-Womdim *et al.* (2001)
Zucchini yellow mosaic virus	Zucchini squash, muskmelon, cucumber and watermelon	The leaf symptoms include mosaic, yellowing, shoestring, and stunting. Fruits are deformed, twisted, and covered with protuberances	Nono-Womdim *et al.* (2001)

Table 2.1 Contd…

Name of the disease	Affected part/Plant	Symptoms	Reference
Chilli veinal mottle virus	Tomato	Yellow foliar mosaic or chlorotic spots	Nono-Womdim *et al.* (2001)
Potato virus Y	Pepper	Mild to severe leaf mosaic, vein-banding or vein-clearing, mosaic patterns on fruits, and plant stunting	Nono-Womdim *et al.* (2001)
Pepino mosaic virus	Tomato	Distorted leaf development, chlorosis and a yellow mosaic	Martinez-Culebras *et al.* (2002)
Chilli veinal mottle virus	Chili	Dark green mottling and reduced leaf size	Prakash *et al.* (2002)
Pepino mosaic virus	Tomato	Yellow mosaic of the leaves	Pagan *et al.* (2006)
Broad bean wilt virus 2	Alstroemeria	Chlorotic spot, mosaic, malformation, necrosis, necrotic mosaic, necrotic ringspot, necrotic spot, stem necrosis, vein clearing, vein necrosis, yellow spot	Fuji *et al.* (2007)
Cucumber mosaic virus	Alstroemeria	Chlorotic spot, mosaic, malformation, necrosis, necrotic mosaic, necrotic ringspot, necrotic spot, stem necrosis, vein clearing, vein necrosis, yellow spot	Fuji *et al.* (2007)
Youcai mosaic virus	Alstroemeria	Chlorotic spot, mosaic, malformation, necrosis, necrotic mosaic, necrotic ringspot, necrotic spot, stem necrosis, vein clearing, vein necrosis, yellow spot	Fuji *et al.* (2007)
Pepino mosaic virus	Tomato	Leaf bubbling, yellow spots, stem necrosis, leaf necrosis	Hanssen *et al.* (2008)
Telosma mosaic virus	Telosma	Puckering, mottling, mosaic and stunting	Ha *et al.* (2008)
Peace lily mosaic virus	Peace lily	Puckering, mottling, mosaic and stunting	Ha *et al.* (2008)
Wild tomato mosaic virus	Wild tomato	Puckering, mottling, mosaic and stunting	Ha *et al.* (2008)

Table 2.1 Contd…

Name of the disease	Affected part/Plant	Symptoms	Reference
Sweet potato feathery mottle virus	Sweet potato	Leaf mosaic	Untiveros *et al.* (2008)
Pepino mosaic virus	Tomato	Nettle-heads, dwarfing, leaf distortions, leaf mosaics, yellow leaf spots, marbling or flaming of fruit	Hanssen *et al.* (2009)
Dasheen mosaic virus	*Colocasia esculenta*	Whitish feathery on leaves	Babu *et al.* (2010)
Zucchini yellow mosaic virus	Zucchini squash	Leaf curl and leaf mosaic	Desbiez *et al.* (2010)
Pepino mosaic virus	Tomato	Yellow mosaic symptoms on leaves	Hasiow-Jaroszewska *et al.*(2010)
Potato virus Y	Tobacco	Systemic veinal necrosis	Tian *et al.* (2011)
Rose rosette virus	*Rosa multiflora*	Leaf proliferation at nodes, red pigmentation of stems and leaves, multiple shoots emerging from a single node to form witches' broom and malformed leaves	Laney *et al.* (2011)
Papaya ringspot virus	Cucurbita moschata	Mosaic, green vein banding	Owolabi *et al.* (2011)
Cowpea aphid-borne mosaic virus	Passion fruit	Chlorotic spots; malformation, crinkle, and mosaic; leaf roll and mosaic	Ochwo-Ssemakula *et al.* (2012)
Cucumber mosaic virus	Apricot	Leaf mottling, marginal leaf necrosis deformation	Ahmed and Fath-Allah (2012)
Plum pox virus	Apricot	Leaf mottling, marginal leaf necrosis deformation	Ahmed and Fath-Allah (2012)
European mountain ash ringspot-associated virus	*Sorbus aucuparia*	Chlorotic ringspots and mottling on leaves	Mielke-Ehret *et al.* (2012)
Alternanthera mosaic potexvirus	*Portulaca* sp.	Chlorotic blotch and irregular leaf margins	Baker and Williams (2013)
Potato virus X	Potato	Blistering and malformation of leaves	Massumi *et al.* (2014)
Potato virus X	*Nicotiana glutinosa*	Mild mosaic and vein clearing	Massumi *et al.* (2014)

Table 2.1 Contd…

Name of the disease	Affected part/Plant	Symptoms	Reference
Turnip mosaic virus	Turnip	Dark green islands, dark green vein banding	Schwinghamer *et al.* (2014)
Peanut mottle virus	Peanut leaves	Dark green patches on a peanut leaves	Soumya *et al.* (2014)
Papaya ringspot virus	Zucchini	Shoestring symptoms and deformed fruits	Ibaba *et al.* (2015)
European mountain ash ringspot-associated virus	*Sorbus aucuparia*	Mottling, chlorotic ringspots and decline of the whole plant	Robbach *et al.* (2015)
Turnip mosaic virus	*Brassica* sp.	Mosaic, mottling, interveinal chlorosis, irregular chlorotic patches and puckering	Singh *et al.* (2015)
Watermelon mosaic virus	Melon ,squash and watermelon	Mosaic, deformation, blistering of leaves and fruits	Ayazpour and Vahidian (2016)
Rose rosette virus	Rose	Witches' broom, (excessive thorn proliferation, abnormal growth of leaves from the flower, excessive stem growth and unusual reddening of leaves, branch proliferation and distorted leaves, distorted leaf and flower	Babu *et al.* (2016)
Watermelon mosaic virus 2	Pumpkin leaf, squash fruit	Dark green mottling, leaf distortion, abnormal leaf shapes on pumpkins yellowing, deformation of squash fruit	Shevchenko *et al.* (2016)
Zucchini yellow mosaic virus	Squash leaf, pumpkin fruit	Filamentary and dark green mosaic on squash leaf, knobs on pumpkin fruit	Shevchenko *et al.* (2016)
High Plains wheat mosaic virus	Wheat	Leaf Mosaic	Alemandri *et al.* (2017)
Pepino Mosaic Virus	Tomato	Yellow spots scattered throughout the leaflet	Bibi *et al.* (2017)
Endive necrotic mosaic virus	Lettuce and chicory	Mosaic or strong necrotic symptoms	Desbiez *et al.* (2017)
Blackberry yellow vein disease	Blackberry	leaf mottling, chlorotic ringspots and curved midribs	Hassan *et al.* (2017)

Table 2.1 Contd…

Name of the disease	Affected part/Plant	Symptoms	Reference
Pigeonpea sterility mosaic virus	Pigeonpea	Seedling showing chlorosis and mosaic symptoms and typical crinkled emerging trifoliate with mosaic symptoms	Kumar *et al.* (2017a)
Cucumber green mottle mosaic virus	Watermelon, melon, and oriental melon	Mosaic and mottle symptoms.	Park *et al.* (2017)
Cucurbit vein banding virus	Squash plant leaf	Strong vein banding	Perotto *et al.* (2018)
Cucurbit vein banding virus	Squash leaf	Strong vein banding	Perotto *et al.* (2018)
Cucumber mosaic virus	*Nicotiana tabacum* cv. *Samsun*	Severe chlorosis symptoms, mild chlorosis symptoms, or vein-clearing symptoms	Qiu *et al.* (2018)

2.4. Important characteristics of *Potyvirus*, *Potexvirus* and *Emaravirus*

2.4.1. *Potyvirus*

The genus *Potyvirus* is the largest genus of the family *Potyviridae*, with nearly 160 definite and tentative species (Berger *et al.*, 2005; Wylie *et al.*, 2017). It was named after the type species *Potato virus Y*. The family *Potyviridae* consists of 8 definite and 2 unassigned genera with 193 definite species (Wylie *et al.*, 2017). It is one of the major group of viruses that infect agricultural, pasture, horticultural and ornamental plants (Ward and Shukla, 1991) and caused major damage in crops worldwide leading to huge financial losses (Martinez *et al.*, 2016). The ICTV recognized *Potyviridae* as the second largest plant virus family after *Geminiviridae* (Ivanov *et al.*, 2014). They infect a wide range of mono- and dicotyledonous plant species and have been found in all parts of the world (Gibbs and Ohshima, 2010).

2.4.1.1. Virion properties

Virions of potyviruses are 680 to 900 nm in length and 11 to 13 nm wide and encapsidate a monopartite, single-stranded RNA genome of ~10kb. Sedimentation coefficient and extinction coefficient of virions were reported to be $S_{20,W}$=137-160S and $E^{0.1\%}_{1cm,\ 260\ nm}$=2.4-2.7 respectively and density in cesium chloride (CsCl) were 1.31 g cm^{-3} (Wylie *et al.*, 2017). A unique feature shared by all potyviruses is the induction of characteristic pinwheel or scroll-shaped inclusion bodies in the cytoplasm of the infected cells (Edwardson, 1974). These cylindrical inclusion bodies are formed by a virus-encoded protein and can be considered as the most important phenotypic criterion for assigning viruses to the *Potyvirus* group (Milne, 1988; Shukla *et al.,* 1989). The single coat or capsid protein (CP) of potyviruses of 30-47 kDa was derived from the C-terminus of the polyprotein and belongs to a large family of plant virus CPs forming filamentous capsids (Dolja *et al.*, 1991; Allison *et al.*, 1986; Domier *et al.*, 1986; Wylie *et al.*, 2017). The CP is a three domain protein with variable N- and C-terminal regions and it is exposed on the virion surface and is susceptible to mild treatment with trypsin. The central domain forms the core subunit structure (Allison *et al.*, 1985; Shukla *et al.*, 1988).

2.4.1.2. Genome organization

The *Potyvirus* genome consists of a single stranded, positive sense RNA molecule of ~9.7 kb. The organization of the 5′ end of the genome carries a viral protein genome linked protein VPg and a poly-A tail covalently bound to the 3′ end. The genome (Fig. 2.1) contains a single long open reading frame (ORF) which is translated into a large 340-370 kDa polyprotein and then cleaved by virus-encoded proteinases into ten functional proteins: N-terminal protein (P1 protein), helper component protein (HC-Pro), P3 protein, 6kDa protein 1 (6K1), cytoplasmic inclusion protein (CI), 6kDa protein 2 (6K2), nuclear inclusion A linked VPg protein (NIa-VPg), nuclear inclusion protein A (NI-a), nuclear inclusion protein B (NI-b) and capsid or coat protein (CP) (Riechmann *et al.*, 1992; Shukla *et al.*, 1994; Trigiano *et al.*, 2003; Ivanov *et al.*, 2014; Wylie *et al.*, 2017). Tatineni *et al.* (2009)

reported a second ORF within the P3 coding region. This ORF encodes another protein called P3-Pretty Interesting Potyviridae ORF (P3N-PIPO) from a translational frameshift.

2.4.1.3. Taxonomy

The genus *Potyvirus* consists of 160 species as mentioned earlier where less than 76% nucleotide identity and less than 82% amino acid identity of the complete ORF determined the species demarcation criteria. However, for coat protein coding region the demarcation criteria was 76-77% nucleotide identity and 80% amino acid identity (Adams *et al.*, 2005; Wylie *et al.*, 2017).

2.4.1.4. Transmission

Potyviruses were reported to be transmitted by aphids (Subfamily: Aphidinae, Genera: *Macrosiphum* and *Myzus*) in non-persistant manner and by mechanical sap inoculation. However, few isolates were reported not to be transmitted by aphids (Gibbs *et al.*, 2003; Poutaraud *et al.*, 2004; Fauquet *et al.*, 2005). Some species were also known to be seed transmissible (Johansen *et al.*, 1994) and some were reported to be transmitted by tubers also (Shukla *et al.*, 1994).

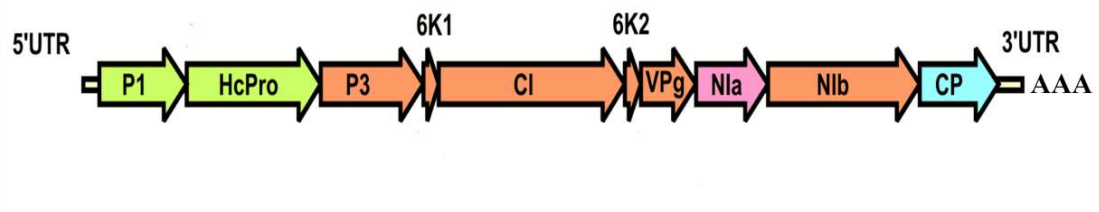

Fig. 2.1: Genome organization of *Potyvirus* showing different proteins coding regions: N-terminal protein (P1 protein), helper component protein (HC-Pro), P3 protein, 6 kDa protein 1 (6K1), cytoplasmic inclusion protein (CI), 6 kDa protein2 (6K2), nuclear inclusion A linked VPg protein (NIa-VPg), nuclear inclusion protein A (NI-a), nuclear inclusion protein B (NI-b), coat protein (CP).

2.4.2. *Potexvirus*

The genus *Potexvirus* belongs to the family *Alphaflexiviridae* which contains 7 definite and one unassigned genera. The genome comprises of single stranded positive sense RNA. The virus was named after the type species *Potato virus X*. Different species of the genus can infect both monocot and dicot plants (Martelli *et al.*, 2007; Website reference 2, https://talk. ictvonline.org/files/master-species-lists/m/msl/6776).

2.4.2.1. Virion properties

Virions of potexviruses are 470 to 580 nm in length and 12 to 13 nm wide and encapsidate a monopartite, single-stranded RNA genome of 5.9 to 7.0 kb. The virion is encapsidated with a single coat protein of 22-27 kDa. Sedimentation coefficient of virion was reported to be $S_{20,W}$=115-130S and density in cesium chloride (CsCl) were 1.31 g cm^{-3} (Adams *et al.*, 2004; Martelli *et al.*, 2007; Verchot-Lubicz *et al.*, 2007; Kim *et al.*, 2010).

2.4.2.2. Genome organization

Potexvirus genome (Fig. 2.2) encodes five ORFs (RNA dependent RNA polymerase (RdRP) gene, three triple gene block (TGB1, TGB2 and TGB3) genes and a coat protein (CP) gene (Adams *et al.*, 2004; Verchot-Lubicz, 2007; Kim *et al.*, 2010). The 5′ end of the genome consists of methylguanosine cap with a 3′ poly (A) tail (Huisman *et al.*, 1988; Huang *et al.*, 2004; Martelli *et al.*, 2007). The RdRP encodes the viral polymerase that helps in virus replication. TGB proteins help in cell-to-cell movement of the virus (Verchot-Lubicz, 2005; Martelli *et al.*, 2007), whereas, the CP is required for virion assembly and virus cell-to-cell movement (Huisman *et al.*, 1988; Santa Cruz *et al.*, 1998; Martelli *et al.*, 2007).

2.4.2.3. Taxonomy

Potexvirus currently consists of 38 species. The species demarcation criteria for the viruses belongs to this genus was determined by less than 72% nucleotide identity or less than 80% amino acid identity of the CP or RdRP gene (Adams *et al.*, 2004; Kim *et al.*, 2010).

2.4.2.4. Transmission

In general potexviruses can infect a wide range of plant species of both monocot and dicot. But, individual species of the genus showed narrow host range. All the members of the genus were reported to be transmitted by mechanical means. Recently *Bamboo mosaic virus* (BaMV, Genus: *Potexvirus*) RNA was detected inside two insect vectors- *Gastrozona fasciventris* and *Atherigona orientalis* (Order: Diptera) during insect transmission studies where the researchers suggested that the virus transmission might happened in mechanical-like manner (Chang *et al.*, 2017).

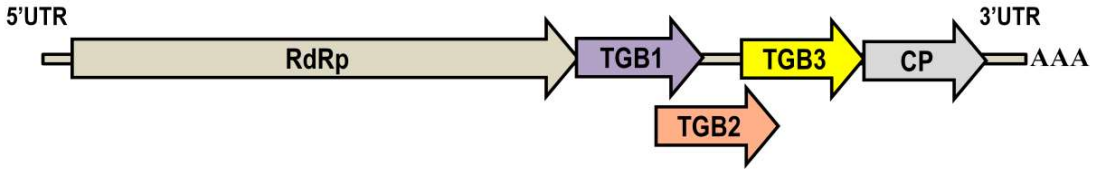

Fig. 2.2: Genome organization of *Potexvirus* showing different protein coding regions: RNA dependent RNA polymerase (RdRp), Triple gene block 1 (TGB1), Triple gene block 2 (TGB2), Triple gene block 3 (TGB3), coat protein (CP).

2.4.3. *Emaravirus*

The genus *Emaravirus* (Family: *Fimoviridae*) is a multipartite (segmented) negative sense single stranded RNA virus and named after the type species *European mountain ash ringspot-associated virus*. It is related to members of the family *Bunyaviridae* (Mielke-Ehret and Muhlbach, 2012; Website reference 2, https://talk.ictvonline.org/files/master-species-lists/m/msl/6776).

2.4.3.1. Virion properties

Virion shows approximately spherical double membrane bodies (DMBs) of 80-200 nm diameter or sometimes filamentous bodies encapsidating 1.4-7.0 kb segmented genome. The viral envelop was reported to be composed of mainly 35.1 kDa nucleocapsid protein with integrated glycoprotein

(Mielke-Ehret and Muhlbach, 2012; Elbeaino *et al.*, 2013; Hassan *et al.*, 2017).

2.4.3.2. Genome organization

Emaravirus genome is characterized by three to seven negative sense multipartite RNA particles. Each segment of RNA codes for one protein. RNA 1 codes for a single RNA dependent RNA-polymerase (RdRp) of 264-269 kDa (Lukashevich *et al.,* 1997; Bruenn, 2003; Mielke-Ehret and Muhlbach, 2012). RNA 2 codes for a glycoprotein precursor, p2 of 73-75 kDa having six potential sites for N-glycosylation which forms a homodimer. The glycoprotein precursor is predicted to be cleaved into two products of 52 kDa and 23 kDa (Garian, 2001; Mielke-Ehret and Muhlbach, 2012). RNA 3 encodes a 32-35 kDa nucleocapsid protein (Garian, 2001; Mielke-Ehret and Muhlbach, 2012). RNA 4 encodes a closteroviral Hsp70h-like protein of 27-42 kDa that helps in cell-to-cell movement (Dolja *et al.,* 2006; Laney *et al.,* 2011; Mielke-Ehret and Muhlbach, 2012). Three more segments of varying sizes and unknown function have been discovered (Mielke-Ehret and Muhlbach, 2012; Di Bello *et al.,* 2015; Hassan *et al.*, 2017; Babu *et al.*, 2017). However, in case of different species different numbers of RNA segments were noticed (Mielke-Ehret and Muhlbach, 2012).

2.4.3.3. Taxonomy

Emaravirus currently consists of 9 species viz., *Actinidia chlorotic ringspot-associated virus* (AcCRaV; Zheng *et al.*, 2017), *European mountain ash ringspot-associated virus* (EMARaV; Mielke-Ehret and Muhlbach, 2012), *Fig mosaic virus* (FMV; Elbeaino *et al.*, 2009), *High Plains virus* (HPV; Mielke-Ehret and Muhlbach, 2012), *Wheat mosaic virus* (WMoV; Tatineni *et al.*, 2014), *Pigeonpea sterility mosaic virus 1* (PPSMV-1; Elbeaino *et al.*, 2014), *Pigeonpea sterility mosaic virus 2* (PPSMV-2; Elbeaino *et al.*, 2015), *Raspberry leaf blotch virus* (RLBV; McGavin *et al.*, 2012), *Redbud yellow ringspot-associated virus* (RYRSaV; Laney *et al.*, 2010) *and Rose rosette virus* (RRV; Laney *et al.*, 2011; Di Bello *et al.,* 2015). *Emaravirus* shared similarity with *Bunyaviridae* (Mielke-Ehret and Muhlbach, 2012).

2.4.3.4. Transmission

The members of *Emaravirus* are readily transmitted by eriophyid mites including *Phytoptus pyri* (EMARaV; Mielke-Ehret *et al.*, 2010), *Phyllocoptes fructiphylus* (RRV; Amrine *et al.*, 1988), *Phyllocoptes gracilis* (RLBV; Jones *et al.*, 1984) *Aceria tosichella* (HPV; Seifers *et al.*, 1997), *Aceria cajani* (PPSMV; Seth, 1962; Kulkarni *et al.*, 2002) and *Aceria ficus* (FMV; Flock and Wallace, 1955). However, AcCRaV was also sap transmissible (Zheng *et al.*, 2017).

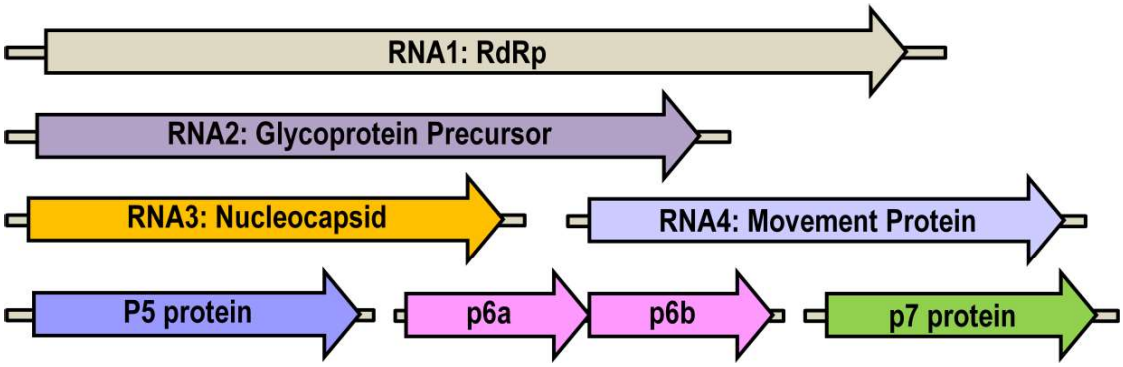

Fig. 2.3: Genome organization of *Emaravirus* showing different proteins coding RNA particles: RNA dependent RNA polymerase (RdRP), glycoprotein precursor, necleocapsid, movement protein and three RNA particles (p5, p6 and p7) of unknown function.

2.5. Insect vectors

Transmission of a plant virus between host plants within a field or between distant fields is often dependent on a vector. To infect a plant, a virus must physically penetrate the cell wall barrier, which may result from mechanical inoculation carried out by humans or natural transmission by animals, insects, nematodes or fungi (Khan and Dijkstra, 2002; Trigiano *et al.*, 2003). Insects are the most important vectors of plant viruses because of their abundance and feeding behaviour (Harris, 1991). About 70% of plant viruses have insect vectors, more than 50% of which are homopterans (Francki *et al.*, 1991). A small number of viruses can be transmitted through pollen to seeds (e.g., *Barley stripe mosaic virus*, genus: *Hordeivirus*), while many of them cause systemic infection, which

accumulate in vegetatively propagated crops. Transmission of the viruses through insect vectors might depend upon feeding manner and retention in vectors and are categorized into four types: (a) Non-persistent transmission- Stylet-borne; (b) Semi-persistent transmission- Foregut borne and intermediate retention; (c) Persistent circulative transmission- Replicate only in plants; (d) Persistent propagative transmission- Able to replicate in both plants and insect vector (Watson and Roberts, 1939; Sylvester, 1956; Sastry, 2013; Roossinck, 2015; Whitfield *et al.*, 2015). Some vectors of major plant RNA viruses along with their mode of transmission have been presented in the Table 2.2.

Table 2.2. Insect vectors of *Potyvirus*, *Potexvirus* and *Emaravirus* along with their mode of transmission

RNA Virus*	**Vector**	**Insect vector group**	**Mode of transmission**	**Reference**
Potyvirus				
PRSV	*Myzus persicae*	Aphids	Non circulative helper strategy	Sanchez and Martinez (1977)
PRSV	*Myzus persicae*	Aphids	Non circulative helper strategy	Makkouk and Lesemann (1980)
PRSV	*Aphis gossypii*	Aphids	Non circulative helper strategy	Yemewar and Mali (1980)
PRSV	*Myzus persicae, Aphis gossypii, Aphis neeri, Aphis citricola* and *Raphalosiphum maidis*	Aphids	Non circulative helper strategy	Teliz *et al.* (1991)
CMV, PRSV and TRSV	*Myzus persicae*	Aphids	Non circulative helper strategy	Lecoq (1992)
CMV, PRSV and ZYMV	*Myzus persicae*	Aphids	Non circulative helper strategy	Brown *et al.* (1993)
PRSV and PLRV	*Myzus persicae*	Aphids	Non circulative helper strategy	Souza-Dias *et al.* (1993)
PRSV	*Aphis gossypi* and *Myzus persicae*	Aphids	Non circulative helper strategy	Chao *et al.* (1994)
PRSV	*Myzus persicae* *Aphis gossypii, Aphis craccivora* and *Pentalonia nigronervosa*	Aphids	Non circulative helper strategy	Shaikh (1996)

Table 2.2 Contd…

RNA Virus*	Vector	Insect vector group	Mode of transmission	Reference
PRSV	*Myzus persicae*, *Aphis gossypii* and *Aphis craccivora*	Aphids	Non circulative helper strategy	Reddy (2000)
PRSV	*Aphis gossypii, Lipaphis erysmi, Myzus persicae* and *Toxoptera citricidus*	Aphids	Non circulative helper strategy	Giampan and Rezende (2001)
PRSV	*Myzus persicae, Aphis gossypii* and *Aphis craccivora*	Aphids	Non circulative helper strategy	Reddy *et al.* (2007)
PVY^{O}, $PVY^{N:O}$ or PVY^{NTN}	*Myzus persicae*	Aphids	Non circulative helper strategy	Mondal and Gray (2017)
Potexvirus				
PAMV	*Myzus persicae*	Aphids	Non circulative	Fereres and Raccah (2015)
BaMV	*Gastrozona fasciventris* and *Atherigona orientalis*	Aphids	Non circulative	Chang *et al.* (2017)
Emaravirus				
FMV	*Aceria ficus*	Mites	Circulative-propagative	Flock and Wallace (1955)
PPSMV	*Aceria cajani*	Mites	Circulative-propagative	Seth (1962)
RLBV	*Phyllocoptes gracilis*	Mites	Circulative-propagative	Jones *et al.* (1984)
RRV	*Phyllocoptes fructiphylus*	Mites	Circulative-propagative	Amrine *et al.* (1988)
HPV	*Aceria tosichella*	Mites	Circulative-propagative	Seifers *et al.* (1997)
PPSMV	*Aceria cajani*	Mites	Circulative-propagative	Kulkarni *et al.* (2002)
EMARaV	*Phytoptus pyri*	Mites	Circulative-propagative	Mielke-Ehret *et al.* (2010)
RRV	*Phyllocoptes fructiphylus*	Mites	Circulative-propagative	Di Bello *et al.* (2015)

***** CMV= *Cucumber mosaic virus*, PRSV= *Papaya ringspot virus*, TRSV= *Tobacco ringspot nepovirus*, ZYMV= *Zucchini yellow mosaic virus*, PLRV= *Potato leafroll virus*, PVY= *Potato virus Y*, PAMV= *Potato aucuba mosaic virus*, BaMV= *Bamboo mosaic virus*, EMARaV= *European mountain ash ringspot-associated virus*, RRV= *Rose rosette virus*, RLBV= *Raspberry leaf blotch virus*, HPV= *High Plains virus*, PPSMV= *Pigeonpea sterility mosaic virus*, FMV= *Fig mosaic virus*.

2.6. Occurrence of plant diseases caused by selected RNA viruses

2.6.1. *Potyvirus*

During the late 1920s, Kenneth Smith detected *Potato virus Y* while studying potato viruses in the United Kingdom (Smith, 1931). Subsequently, other viruses were shown to have similar properties, and in 1971 this group was given the acronym of "*Potyvirus*" for an experimental period of five years (Harrison *et al.,* 1971). A breakthrough in *Potyvirus* studies was achieved when the complete genome sequences *Tobacco etch virus* (TEV; Allison *et al.*, 1986), *Tobacco vein mottling virus* (TVMV; Domier *et al.*, 1986), *Potato virus Y* (PVY; Robaglia *et al.,* 1989), *Plum pox virus* (PPV; Lain *et al.*, 1989a; Maiss *et al.*, 1989; Teycheney *et al.*, 1989; Riechmann *et al.*, 1990) and *Zucchini yellow mosaic virus* (ZYMV; Gal-On *et al.,* 1991) were reported for the first time. Intensive research during the following years led to a greater understanding of the potyviral genome structure and expression. *Papaya ringspot virus* (PRSV) was reported from Hawaii causing epidemic in papaya (Gonsalves and Ishii, 1980). Later on, PRSV was reported from different continents of the world infecting papaya (Bayot *et al.,* 1990; Thomas and Dodman, 1993; Bateson *et al.,* 1994; Wang and Yeh, 1997; Jain *et al.,* 1998; Bateson *et al.,* 2002; Jain *et al.,* 2004; Davis *et al.,* 2005; Chin *et al.,* 2007; Tripathi *et al.,* 2008; Mansilla *et al.,* 2013) and cucurbits (Mohammed *et al.,* 2012; Mansilla *et al.,* 2013; Ibaba *et al.,* 2015). *Zucchini yellow mosaic virus* (ZYMV) caused an epidemic in Hungary in 1995 (Tobias *et al.*, 1996). PRSV, ZYMV, *Cucumber mosaic virus* (CMV), *Cucumber necrosis virus* (CuNV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus-2* (WMV-2) and *Tomato spotted wilt virus* (TSWV) were also reported to be associated with the cucurbits during 2002-2004 in Iran (Massumi *et al.*, 2007). ZYMV in cucumber, pumpkin, squash and melon was widespread in the farms of KwaZulu-Natal, South Africa (Usher *et al.*, 2012).

Although occurrence of potyviral diseases have been reported throughout the world, according to Shukla *et al.* (1998) their prevalence in the tropical and subtropical countries are of much concern. In India, about

55 *Potyvirus* species have been reported so far (Sharma *et al.*, 2014). PRSV was reported to be a major threat to papaya and cucurbit production in India for several years (Mishra and Jha, 1955; Garga, 1963; Khurana and Bhargava, 1970; Surekha *et al.,* 1977; Yemewar and Mali, 1980; Cheema and Reddy, 1985; Susan, 1985; Jain *et al.*, 2004; Saha *et al.,* 2014). *Peanut stripe potyvirus* was reported as a major pathogen of peanut in India (Jain *et al.*, 2000). *Pepper veinal mottle virus* infection in chili caused considerable loss in Indian economy (Nagaraju and Reddy, 1980). *Tobacco streak virus* infecting soybean was also prominent in India (Kumar *et al.*, 2008). PRSV in snake gourd was reported from Tamil Nadu during 2008-2009 (Kumar *et al.*, 2014).

2.6.2. *Potexvirus*

Potexvirus was named after the type species *Potato virus X* (PVX) and was reported to infect potato in United Kingdom (Smith, 1931) and other potato growing areas (Bercks, 1970; Beemster and de Bokx, 1987; Jones, 1982; Chabbouh, 1989; Brunt and Loebenstein, 2001). It was first introduced in the family *Flexiviridae* in 2004 (Adams *et al.*, 2004). There are a few reports of potexviruses that naturally infect cucurbits. *Trichosanthes virus* (TV) was isolated in 1988 from *Trichosanthes dioica* in the United States (Purcifull *et al.*, 1988; 1999). *Alternanthera mosaic virus* (AltMV) was firstly reported to infect *Alternanthera* from Australia (Geering and Thomas, 1999). Later it was also reported to infect some other crops in United States, Europe, Brazil and Asia (Hammond and Reinsel, 2015; Iwabuchi *et al.*, 2016). *Pepino mosaic virus* (PepMV) was reported to infect pepino in 1980 (Jones *et al.*, 1980). Later on, it was also reported to infect tomato from several continents of the world (Mumford and Metcalfe, 2001; Martinez-Culebras *et al.*, 2002; Pagan *et al.*, 2006; Hanssen *et al.*, 2008; 2009; Hasiow-Jaroszewska *et al.*, 2010; Bibi *et al.*, 2017). Recently, *Lagenaria mild mosaic virus* (LaMMoV) has also been reported as a pathogen of bottle gourd in Mayanmar (Kim *et al.*, 2010). In India, PVX was reported to infect brinjal and potato (Kumar *et al.*, 2016; Sharma *et al.*, 2016).

2.6.3. *Emaravirus*

The genus *Emaravirus* was named after the type species *European mountain ash ringspot-associated virus* (EMARaV) that was reported for the first time to infect European mountain ash in Germany (Benthack *et al.*, 2005). EMARaV was also reported from North and Central Europe (Robbach *et al.*, 2015). Other species of the genus like- HPV, FMV (Elbeaino *et al.*, 2009; Caglayan *et al.*, 2010; Ishikawa *et al.*, 2012; Mahmoud *et al.*, 2014), PPSMV (Elbeaino *et al.*, 2013; Elbeaino *et al.*, 2014 Elbeaino *et al.*, 2015) and RLBV (McGavin *et al.*, 2012; Quito-Avila and Martin, 2012) were also reported from several continents of the world. The most studied member of this genus is *Rose rosette virus* (RRV). In 1940, it was first reported as infecting roses from Canada (Conners 1941). In 1941, similar symptoms were also reported in California and Wyoming in the USA (Thomas and Scott, 1953). Later, the disease was reported from other regions of the USA such as Florida (Laney *et al.,* 2011; Babu *et al.,* 2014; Baker *et al.,* 2014; Babu *et al.*, 2017). Recently, PPSMV was reported to infect pigeonpea in India (Kumar *et al.*, 2017a).

2.7. Virus detection methods and disease diagnosis

Correct identification of virus pathogen infecting the plant is the most important criteria for proper control of the disease. Procedures for diagnosis must be accurate; often multiple methods are to be used to avoid any discrepancy. Additionally, quick and cheap methods are always preferable. There are two main procedures for diagnosis of viral pathogens in plants. One is based on serological techniques while the other follow molecular biology procedures based on nucleic acid analysis. Electron microscopy can be very useful in demonstrating the presence of virus infections and preliminary identification of some virus families and genera. However, some virus taxa share similar particle morphology. For example, viruses belonging to the *Flexiviridae, Potyviridae, Closteroviridae* are all flexuous filamentous. In addition, small spherical viruses and viruses in low titer are often missed when examining sap extracts from virus infected plants (Matthews, 1991).

2.7.1. Serological techniques

Serological detection systems are the most powerful tool for detection of viruses and used for more than half a century. In serological methods specific antibodies are developed in animal system that responds to specific antigens (Torrance, 1998). Many serological methods have been reported to be used for virus detection such as: enzyme-linked immunosorvent assay (ELISA), tissue blot immunoassay (TBIA) and quartz crystal microbalance immunosensors (QCMI) (Jeong *et al.*, 2014).

2.7.1.1. ELISA

ELISA is highly sensitive, simple, fast method for the detection of viruses and most importantly can quantify the virus content in plant tissue. Detection of *Plum pox virus* (PPV) and *Arabis mosaic virus* (ArMV) by ELISA was a breakthrough in virus diagnostics (Clark and Adams, 1977). The most frequently used ELISA techniques for detection of plant virus diseases were indirect ELISA, plate-trapped antigen technique (PTA-ELISA) and immune virus particle precipitation ELISA (IP-ELISA) (Dorokhov and Komarova, 2016). During last three decades several plant viruses including CMV, *Citrus tristeza virus* (CTV), PLRV, PVX and PVY (El-Araby *et al.*, 2009; Sun *et al.*, 2001) have been detected using different ELISA techniques. *Potato virus S* (PVS), *Strawberry latent ringspot virus* (SLRSV), PLRV, PVY and PVS were also detected by DAS-ELISA (Wang *et al.,* 2016b; EL-Morsy *et al.,* 2017). Bruisson *et al.* (2017) tested nine grape vine infecting viruses including *Arabis mosaic virus* (ArMV), *Grapevine fleck virus* (GFkV), *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated viruses* (GLRaV-1, -2, -3), *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Grapevine virus A* (GVA), and *Grapevine virus B* (GVB) by ELISA. Recently, Beck *et al.* (2018) reported *Pea seed-borne mosaic virus* (PSbMV) from field peas in North Dakota using DAS-ELISA.

2.7.1.2. TBIA

Like ELISA, TBIA has been proved a reliable virus detection tool for detection of plant viruses (Hancevic *et al.*, 2012). The main difference is

that in case of ELISA a polystyrene plate is used as platform, whereas TBIA is performed on nitrocellulose and nylon membranes (Webster *et al.*, 2004). TBIA has been applied for detection of a number of viral diseases caused by BaMV, CTV, *Tomato spotted wilt virus* (TSWV), PRSV, *Bean yellow mosaic virus* (BYMV), *Sweet potato feathery mottle virus* (SPFMV) and *Cymbidium mosaic virus* (CyMV) (Bove *et al.*, 1988; Lin *et al.*, 1990; Webster *et al.*, 2004; Makkouk and Kumari, 2006; Eid *et al.*, 2008; Shang *et al.*, 2011; Hancevic *et al.*, 2012).

2.7.1.3. QCMI

The quartz crystal microbalance (QCM) technique is widely used to measure small masses in vacuum, gas and liquid condition based on vibrations and frequency change in real time (Kurosawa *et al.*, 2006; Mecea, 2005; 2006). Immunological combination with QCM results in QCMI as a mass-sensitive transducer device (Owen *et al.*, 2007). The antibody-antigen binding reaction causes a decreased quartz crystal oscillation frequency in positive reaction. QCMI has many advantages such as high sensitivity, real time output, portability, label-free entities and low cost of operation, fabrication and maintenance for which it becomes attractive alternatives to conventional analysis methods (Chen and Tang, 2007; Tang *et al.*, 2006). QCMI shows high detection sensitivity for different viruses (Bachelder *et al.*, 2005; Eun *et al.*, 2002; Su *et al.*, 2003; Susmel *et al.*, 2000; Uttenthaler *et al.*, 2001). The QCM instrument coated with virus-specific antibodies is used in detection of plant viruses (Becker and Cooper, 2011; Eun *et al.*, 2002). Several plant viruses including TMV, CyMV, and *Turnip yellow mosaic virus* (TYMV) were detected using QCM (Eun *et al.*, 2002; Dickert *et al.*, 2004; Zan *et al.*, 2012).

2.7.2. Nucleic acid based techniques

Several nucleic acid based techniques have been used for plant virus identification and characterization throughout the world. Some molecular methods which are widely used for detection are fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR) and reverse

transcription PCR (RT-PCR), real-time PCR (qPCR), nucleic acid sequence-based amplification (NASBA) and DNA fingerprinting (Webster *et al.*, 2004).

2.7.2.1. PCR and reverse transcription PCR (RT-PCR)

PCR is a scientific technique used to amplify millions of identical copies of a particular DNA sequence. Now-a-days, PCR is a popular technique for detection of plant viruses in the laboratory and is very commonly used in molecular experiments (Webster *et al.*, 2004; Lopez *et al.*, 2008). RT-PCR technique is sensitive, specific compared to serological methods and is also more reliable than serological methods (Lievens *et al.*, 2005; Lopez *et al.*, 2008). Many potato viruses such as PLRV, PVS and PVX in stem or seeds of potato were detected using this technique (Ham, 2003; Peiman and Xie, 2006; Drygin *et al.*, 2012). Potato viruses within aphids, their vectors, can be detected by RT-PCR (Singh *et al.*, 2004). In addition, RT-PCR was used to detect plant RNA viruses for quarantine purpose (Lee *et al.*, 2011). Five newly reported viruses were identified in Korea including *Cucumber vein yellowing virus* (CVYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Potato aucuba mosaic virus* (PAMV), *Potato yellow dwarf virus* (PYDV), and *Tomato chlorosis virus* (ToCV) using RT-PCR (Lee *et al.*, 2011). A number of plant RNA viruses, *Yam mosaic virus* (YMV), *Peach rosette mosaic virus* (PRMV), *Tomato mottle mosaic virus* (ToMMV), *Tomato chlorosis virus* (ToCV) were detected by RT-PCR technique (Silva *et al.,* 2015; Lee *et al.,* 2016; Ambros *et al.,* 2017; Kang *et al.,* 2018).

2.7.2.2. Multiplex PCR

In multiplex PCR two or more target DNA or RNA can be detected at the same time in a single reaction (Lopez *et al.*, 2008; Webster *et al.*, 2004). In this method several specific primers are required for detection of viruses (Li *et al.*, 2011; Menzel *et al.*, 2002; Singh *et al.*, 2000). Through multiplex-PCR many major characterized viruses were detected from diseased apple trees (Menzel *et al.*, 2002). Yardimci and Culal-Klllc (2011) compared multiplex-PCR and ELISA for the detection of plant viruses, *viz. Apple chlorotic leafspot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), and *Plum pox virus*

(PPV). The authors stated that multiplex-PCR was superior to ELISA in time of detection and in sensitivity. Multiplex RT-PCR technique was used to identify BYMV, *Canna yellow mottle virus* (CaYMV), *Canna yellow streak virus* (CaYSV), *Ranunculus mild mosaic virus* (RanMMV), TSWV and CMV, PVX, PVY, PLRV, *Potato virus* M (PVM) and *Potato virus* A (PVA) (Chauhan *et al.*, 2015; Hayahi *et al.*, 2018; Zhang *et al.*, 2017b).

2.7.2.3. Nested PCR

The method is useful when the virus titre is very low, target gene is unstable and cannot be checked by electrophoresis due to low amplification product (Webster *et al.*, 2004). Several viruses, including CTV, PDV, PPV and PNRSV were detected by this technique (Olmos *et al.*, 1999; Helguera *et al.*, 2001, 2002; Adkar-Purushothama *et al.*, 2011). Nested PCR was combined with immunocapture RT-PCR to increase sensitivity and to simplify preparation of sample (Helguera *et al.*, 2001, 2002). This method was applied to detect *Lettuce mosaic virus* (LMV), TRSV, *Chrysanthemum virus B* (CVB) and *Little cherry virus 1* (LChV1) (Moreno *et al.*, 2007, Lee *et al.*, 2015; Guan *et al.*, 2017; Zhiyong *et al.*, 2017).

2.7.2.4. Co-operational PCR (Co-PCR)

Both co-operational PCR and nested-PCR require a tetra primer set (Olmos *et al.*, 1999; 2002). However, co-operational PCR needs one external and three internal primers instead of two external and two internal primers associated with nested-PCR (Olmos *et al.*, 2002; Pantaleo *et al.*, 2001). Co-operational PCR technique has some benefits over conventional PCR (Lopez *et al.*, 2008; Olmos *et al.*, 2002). *Squash vein yellowing virus* (SqVYV) was detected by using Co-PCR (Adkins *et al.*, 2008). Olmos *et al.* (2002) also used co-operational PCR for detection of *Cherry leafroll virus* (CLRV).

2.7.2.5. Real-time PCR

Real-time PCR is a technique to monitor the amplified products of PCR in real-time and also quantify that product (Ruiz-Ruiz *et al.*, 2007). Real-time PCR has been increasingly used for detection of plant viruses. CTV, TMV and *Citrus leaf blotch virus* (CLBV) were detected and quantified by real-

time PCR (Ruiz-Ruiz *et al.*, 2007; Yang *et al.*, 2012). Different plant viruses infecting cassava plants [*Cassava brown streak virus* (CBSV), *Uganda cassava brown streak virus* (UCBSV), *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV)] were identified using Real-time PCR (Otti *et al.*, 2016). Recently, PVY, *Cotton leaf curl virus* (CLCuV), *Maize Iranian mosaic virus* (MIMV), GFLV, ArMV, GLRaV-1, GLRaV-3 and *Citrus tatter leaf virus* (CTLV) were identified using Real-time PCR (Liat *et al.*, 2017; Shafiq *et al.*, 2017; Ghorbani *et al.*, 2018; Aloisio *et al.*, 2010; Park *et al.*, 2018). The multiplex TaqMan real time RT-PCR was also used for detection and differentiation of *Wheat streak mosaic virus* (WSMV) and *Triticum mosaic virus* (TriMV), GFLV, ArMV, GFkV, GLRaV-1, GLRaV-3, *Sweet potato virus G*, Sweet *potato latent virus* and *Sweet potato mild mottle virus* (Price *et al.*, 2010; Lopez-Fabuel *et al.*, 2013; Lan *et al.*, 2018).

2.7.2.6. Isothermal amplification

Isothermal amplification is an alternative method of sequence specific DNA amplification other than PCR. In this alternative approach DNA can be amplified in a constant temperature and without expensive thermocycler (Yan *et al.*, 2014). A number of isothermal amplification techniques are present such as nucleic acids sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA), helicase-dependent amplification (HDA), invader assay, rolling circle amplification (RCA), recombinase polymerase amplification (RPA), signal mediated amplification of RNA technology (SMART) and nicking enzyme amplification reaction (NEAR) (Yan *et al.,* 2014).

NASBA is a primer-dependent continuous amplification, which works at isothermal condition instead of thermal cycling. The products amplified by NASBA are antisense to the target viral sequences (Lopez *et al.*, 2008). Real-time NASBA (RT-NASBA) has been applied to detect plant viruses including PLRV, *Apple stem pitting virus* (ASPV), *Strawberry vein banding virus* (SVBV) and PPV (Leone *et al.*, 1997; Klerks *et al.*, 2001; Vaskova *et al.*, 2004; Olmos *et al.*, 2007).

Now-a-days, LAMP is a very useful tool of viral detection. In this amplification method, the detection is possible from very low amounts of target gene (Parida *et al.*, 2008). The LAMP assay has been recently applied for the detection of several plant viruses. The reverse transcription LAMP (RT-LAMP) has been developed for detection of RNA viruses such as PVY, PLRV, CMV, *Sugarcane mosaic virus Sorghum mosaic virus*, *Tomato chlorosis virus* (ToCV), *Wheat streak mosaic virus* (WSMV), *Tomato torrado virus* (ToTV), *Rice ragged stunt virus* (RRSV) and *Lily mottle virus* (LMoV), (Nie, 2005; Ju, 2011; Bhat *et al.*, 2013; Keizerweerd *et al.*, 2015; Karwitha *et al.*, 2016; Lee *et al.*, 2015; Lai *et al.*, 2016; Zhao *et al.*, 2018). Recently, for the detection of *Yam mosaic virus* (YMV) infecting yam, a closed-tube reverse transcription loop-mediated isothermal amplification (CT-RT-LAMP) assay was developed (Nkere *et al.*, 2018).

2.7.2.7. Microarray (Oligonucleotide array)

Microarray is an advanced detection method evolved from southern blotting technology. In this technique, instead of nitrocellulose and nylon membrane glass plate is used as a support (Maskos and Southern, 1992) and can differentiate the expression of messenger RNA (Schena *et al.*, 1995). Nam *et al.*, (2014) demonstrated the potential of microarray technique to detect viral pathogens without amplification of viral RNA. This technique has the potential to detect both known and unknown sequences in environmental samples, so it has been proved quite easy to detect unknown viruses by Oligo-chip (Schena *et al.*, 1995; Boonham *et al.*, 2007; Dugat-Bony *et al.*, 2012; Nam *et al.*, 2014). This method was used to detect different cucurbit-infecting plant viruses and potato viruses including PVA, PVM, PVS, PVX, PVY and PLRV (Lee *et al.*, 2003; Bystricka *et al.*, 2005). This method is also used to identify any plant virus and can differentiate relevant strains (Wang *et al.*, 2002; 2003; Zhang *et al.*, 2010; Hammond, 2011). Nam *et al.*, (2014) developed a large-scale oligonucleotide (LSON) chip to identify 538 plant viruses.

2.7.2.8. Next generation sequencing (NGS)

Next generation DNA sequencing techniques are bringing a new era in life science. Now-a-days, it is widely used in detailed analysis of genome of an individual, analysis of RNA transcripts for expression and quantification; profiling of mRNAs, small RNAs, transcription factors, chromatin structure and DNA methylation patterns (Ansorge, 2009). There are number of platforms for analyzing the sequences such as the Roche/454 FLX, the Illumina/ Solexa Genome Analyzer, the Applied Biosystems SOLiD™ System, the Helicos Heliscope™ and Pacific Biosciences SMRT instruments (Mardis, 2008). It is now possible to identify different viruses within metagenomic samples by NGS and widely used for viral detection. Five viruses including PVY and PVS in potato; *Turnip mosaic virus* in rengarenga and in the dock sample, CLRV and a novel virus belonging to the genus *Macluravirus* were identified by NGS (Blouin *et al.,* 2016). Recently, *Ramu stunt virus* infecting sugarcane, GLRaV-3, CTV, *Barley yellow mosaic virus Y* (BaYMV-2), *Barley mild mosaic virus* (BaMMV), CMV, *Chickpea chlorotic dwarf virus* (CpCDV) and *Blackcurrant leaf chlorosis associated virus* (BCLCaV) were identified by NGS (Mollov *et al.,* 2016; Bester *et al.,* 2016; Jooste *et al.,* 2017; Rolland *et al.,* 2017; Kumar *et al.,* 2017b; Zaagueri *et al.,* 2017; James *et al.,* 2018).

2.8. Disease management

Plant viruses are of great concern to the farmers, researchers and policy makers because of the enormous loss they cause in different crops (Waterworth and Hadidi, 1998). Because of their peculiar nature and characteristic association with hosts and vectors, no therapeutic method can completely control them. However, certain preventive measures, if adopted suitably can be of great help in avoiding viral diseases. This includes controlling vectors, avoiding infection sources and maintaining resistance of host plants through exogenous application or transgenic methods. Antiviral substances of plant origin may be used as a component for disease management. Infection of several viral diseases could be

prevented by the application of extracts/antiviral compounds from various plants (Awasthi and Singh, 2015).

2.8.1. Antiviral compounds (secondary metabolites) from plants against viral pathogens

Secondary metabolites in plants play an important role in defense against different pathogens. The most common antimicrobial compound, azadirachtin was obtained from neem. In 2014, Mali and Kachhawa reported the antiviral activity of azadirachtin against yellow vein mosaic virus disease of okra. Essential oil of *Melaleuca alternifolia* was also reported to be effective against TMV (Bishop, 1995). Several other secondary metabolites were also reported to show antiviral activity against several viruses, which includes flavonoid quercetagetin 3'-methylether from *Centaurea rupestris* against *Tomato bushy stunt virus* (Rusak *et al.*, 1997); sesquiterpene hydrocarbons from *Teucrium polium* against CMV (Bezic *et al.*, 2011); beta-caryophyllene and caryophyllene oxide from *Teucrium arduini* against TMV and CMV (Dunkic *et al.*, 2011); sesquiterpene alpha-bisabolol from *Micromeria graeca* against CMV (Vuko *et al.*, 2012); limonene, 1,8-cineole, and α- zingiberene from Chinese aromatic plants against TMV (Lu *et al.*, 2013) and tababiphenyls from tobacco against TMV (Shang *et al.*, 2014).

2.8.2. Proteins or peptides as antiviral compounds of plant origin

Although very little works have been done so far about the antiviral proteins of plant origin against plant viruses, a 16-20 kDa basic glycoprotein (*viz.*, virus inhibitory agent) was identified from *Boerhaavia diffusa* showing antiviral activity in several plants against several viruses (Awasthi and Mukerjee, 1980; Awasthi *et al.*, 1984; 1985; Kempiak *et al.,* 1991; Awasthi and Rizvi, 1998; 1999; Awasthi, 2000; 2002; Singh and Awasthi, 2002; Kumar and Awasthi, 2003a, 2003b; Awasthi *et al.,* 2003). The inhibition of different viruses such as TMV, PVX, PVY, CMV, AMV, ACMV and CaMV by pokeweed antiviral peptide has been reported (Chen *et al.,* 1991). Inhibition of TMV infection and replication by antiviral factor has also been reported (Chen *et al.,* 1991).

2.8.3. Other proteins or peptides as antiviral compounds

Different kinds of antimicrobial proteins (AMPs)/low molecular weight (LMPs) peptides/globular proteins have been reported to inhibit virus diseases. A new strategy for plant virus control using synthetic LMPs of about 50 amino acid residues has been raised since 15 years ago. Peptides could offer a direct interaction against viral proteins involved in virus infection cycle that affects viral replication. Peptidomic, as a new tool, has been evolved, that led to screen and select the best peptide having antiviral activity, and to re-design in enhancing the biological effect to direct control of the disease (Mendoza-Figueroa *et al.,* 2014).

The AMPs may induce salicylic acid signaling pathway, programmed cell death (PCD), and induced systemic resistance (ISR) by activating PR proteins, BTH (benzothiadiazole) and ethylene factor during begomovirus infection (Mandadi and Clothof, 2013; Trejo-Saavedra *et al.,* 2013; Chen *et al.,* 2013; Mase *et al.,* 2013). Specific synthetic peptide inhibitor of TMV infection has been reported by Marcos *et al.* (1995). Therefore, selection and re-designing of suitable antimicrobial proteins or peptides are needed that will help to make a strategy for direct control of plant viral diseases.

2.8.4. *In vivo* application of crude plant extracts

In vivo application of crude plant extracts either as antiviral agents or through controlling viral vectors are being used extensively now-a-days.

2.8.4.1. Direct virus management

Yellow mosaic disease of mug bean, caused by virus, was successfully managed through the direct application of crude leaf extract of *Azadirachta indica, Boerhaavia diffusa* and *Clerodendrum aculeatum* (Singh and Awasthi, 2009). Leaf extracts of *Azadirachta indica* and *Bougainvillea spectabilis* were reported to show significant disease reduction against TMV and *Tomato mosaic tobamovirus* in *N. glutinosa* (Madhusudhan *et al.*, 2011). *Sapindus* and *Solanum* seed extracts were also used in managing chili leaf curl disease (Ahmed and Raghu Ram, 2016). Petrov *et al.* (2016) proposed that the use of >20% methanolic extract of *Tenacetum vulgare*

would be able to inhibit CMV and PVY infection in tomato. Neem leaf extract was found effective in reducing leaf curl and bud necrosis diseases of tomato (Ruth *et al.*, 2016). Viral diseases of watermelon were reported to be reduced by seed treatment followed by foliar spray of *Boerhaavia diffusa* root extract and *Azadirachta indica* leaf extract (Sharma *et al.*, 2017). Bark extract of *Terminalia arjuna* was also seen to reduce leaf curl symptoms of chili, affected with viral disease (Chaubey *et al.*, 2017).

2.8.4.2. Vector management

Insect vectors are one of the sources of virus transmission. According to Groen *et al.* (2017) one vector might be able to transmit more than one type of viruses. They have profound effect on creating epidemics; hence, managing vector might be of much concern in virus transmission. Neem seed kernel extract, karanj and tumba seed extract was found to be effective against whitefly in controlling leaf curl disease of chili (Pandey *et al.*, 2010). Aquous leaf extract of *Azadirachta indica* and *Clerodendrum aculeatum* and root extract of *Boerhaavia diffusa* were also able to control vector-borne *Mungbean yellow mosaic virus* (Singh *et al.*, 2011). Insecticidal activity of ethanolic extract of *Petiveria alliaceae, Trichilia arborea* and *Azadirachta indica* against whitefly was reported (Cruz-Estrada *et al.*, 2013). Asare-Bediaco *et al.* (2014) reported that application of 10% neem leaf or garlic extract was effective in managing okra leaf curl disease by reducing whitefly population. Baldin *et al.* (2015) suggested the use of *Toona ciliate, Trichilia pallida* and *Trichilia casaretti* in managing whitefly vector on tomato.

2.8.5. Induction of defense against viral disease

Several antiviral proteins of plant origin have been reported to inhibit viral pathogens or to induce host systemic resistance. Two antiviral proteins *viz.*, Dianthin 30 and Dianthin 32, from *Dianthus caryophyllus* leaves were reported to show significant inhibitory effect on TMV replication (Stirpe *et al.*, 1981). In 1996, Olivieri *et al.* identified CIP-29 and CIP-34 from *Clerodendrum inerme* leaves that showed systemic host resistance induction activity against viruses by inactivating ribosome. Another

ribosome inactivating protein (RIP) known as Mirabilis antiviral protein (MAP) was found effective in *Mirabilis jalapa* against PVY, PVX, PLRV and *Potato spindle tuber viroid* (Vivanco *et al.,* 1999). Several other RIPs were identified from pokeweed (Park *et al.*, 2002); *Phytolacca americana* and *Boerhaavia diffusa* (Gholizadeh *et al.*, 2004) that showed systemic defense induction in different hosts. Systemic defense induction by RIPs in bean against TNV, in *Chenopodium* against TMV and in *Vicia faba* against BYMV was also reported by Barakat *et al.* (2005). Systemic inhibition of BYMV in *Vicia faba* using *Chrysanthemum cinerariaefolium*, *Clerodendrum inerme*, *Dianthus caryophyllus*, *Mirabilis jalapa*, *Phytolacca Americana* and *Schinus terebinthifolius* extract (leaf, root or young shoot extract) was also reported by Mahdy *et al.* (2007).