REVIEW
OF
LITERATURE
India is one of the largest producers of vegetables and ranks second in the world, next to China with productivity around 45 Mt from 45 million ha. It is especially true that cucurbitaceous crops occupy an area of 500 million hectares with productivity at 8 Mt ha\(^{-1}\). Commercially, about 19 different types of cucurbits are grown in different states. Maharashtra is the fifth largest state in India’s vegetable production, where about 0.21 million ha (Anon. 2002) are under vegetable cultivation. The productivity of the cucurbitaceous crop is severely affected in the last decade by many virus diseases in this state. The viral diseases cause losses through reduction in growth and yield of marketable produce. The viruses emerging as major challenges in India belong to the genera Begomovirus, Cucumovirus, Potyvirus, Tobamovirus and Tospovirus.

The RNA viruses affecting cucurbitaceous crops are *Cucumber green mottle mosaic virus* (CGMMV) and Squash mosaic virus (SqMoV) of the genus *Tobamovirus*. These viruses are either mechanically transmitted or seed borne. Among various Potyviruses, *Papaya ring spot virus* (PRSV), *Watermelon mosaic virus* (WMV-1), *Zucchini yellow mosaic virus* (ZYMV), are affecting cucurbit crops. *Cucumber mosaic virus* (CMV) belongs to *Cucumovirus* is also one of the main virus affecting various cucurbit crops. Both Poty and Cucumoviruses are transmitted by an insect vector *Aphid* spp. In addition to these viruses, the RNA virus, which is *Watermelon bud necrosis virus* (WBNV), which is transmitted by *Thrips* spp., also infects cucurbitaceous crops. In the case of DNA viruses, the viruses belongs to the
genus *Begomovirus* are also economically important, such as *Tomato leaf curl New Delhi Virus* [Luffa-isolate], and *Squash leaf curl china virus* (SLCCNV) which are transmitted by an insect vector *Bemisia tabaci* (Whitefly). However viral diseases would be managed by adopting different prophylactic measures such as use of net-house, pyrethroids and destruction of weed reservoir, strict sanitation around crop premises by destruction of virus reservoir crops these steps would be helpful to a certain extent, but the above practices fails to offer complete solution. The important cucurbit infecting viruses reported from India are *Cucumber green mottle mosaic virus* (Raychoudhari and Verma, 1978), *Cucumber mosaic virus* (Vani, 1987), *Watermelon Mosaic virus* (Raychoudhari and Verma, 1975) and *Watermelon bud necrosis virus* (Jain et al., 1998, Mandal et al., 2003). Apart from the above viral pathogens, Begomoviruses are emerging pathogens in many cucurbit crops in India (Varma and Malathi, 2003).

A large number of viruses have been reported to infect cucurbitaceous crops and many are economically important. Most of the viruses can infect a number of plant species belonging to different genera and families, but a few appear restricted to cucurbitaceae. Twenty six of these viruses are mechanically transmissible in the sap of infected plants, while nine are known to be seedborne. *Cucumber green mottle mosaic virus* is the predominantly occurring pathogen in India, particularly affecting bottlegourd, cucumber, melon and watermelon crops.

**Occurrence and distribution of viral pathogens infecting cucurbits**

**Potyvirus**: The genus *Potyvirus* of the family *Potyviridae* is the major virus group affecting cucurbits, of which *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus*-2 (WMV-2) and *Papaya ring spot virus watermelon isolate* (PRSV-W) are economically important and have world wide distribution (Lecoq *et al.*, 1998).
During the survey of commercial gherkin fields around Kuppam in Andhra Pradesh during 2003, the natural infection of mosaic disease exhibiting mosaic, blistering, vein banding, and malformation of leaves and fruit has been widely observed. About 30-60% yield loss due to viral disease caused mainly by ZYMV, WMV-2 and PRSV-W was reported (Srinivasulu et al., 2010). On the basis of symptomatology, transmission, host range, serological relationship, and particle morphology, the virus causing mosaic disease in gherkin was characteristic of a member of Potyviridae. However, confirmation of taxonomic identity of the virus could be done by molecular characterization (Srinivasulu et al., 2010).

Papaya ringspot virus type W (PRSV-W) formally identified as Watermelon mosaic virus 1 (WMV-1), was found to exist in two distinct entities, which were named as WMV-1 and WMV-2. Recently, WMV-1 and PRSV were found to be serologically identical but were two distinct pathotypes PRSV-P (papaya) and PRSV-W (watermelon) (Srinivasulu et al., 2010). Incidence of PRSV in cucurbits has been documented by virologists in different locations. Walkey et al. (1987) surveyed the incidence of plant viral diseases in economically important vegetable and other crops in Melbourne. They recorded the incidence of PRSV, ZYMV and Melon rugose virus in cucurbits. The mixed infections due to PRSV-W, CMV, and WMV-2 were confirmed by Enzyme Linked Immuno Sorbent Assay (ELISA) in 273 field samples of melon, cucumber, watermelon, zucchini squash, zucchini marrow, pattison and winter squash (Dikova, 1994).

Provvidenti (1996) reported that PRSV generally occurs in tropical and subtropical regions of the world. Recently, an isolate of PRSV-P from Taiwan was found to cause a more severe symptom than the usual one, in cucurbits. Among 10
cucurbitaceous vegetables surveyed by Dahal et al. (1997), more than 68 locations in inner Tarai districts of Nepal had the incidence of PRSV in Benincasa hispida, Momordica charantia, Trichosanthes cucumerina, Luffa acutangula, Cucurbita pepo, Citrullus vulgaris, Lagenaria siceraria and Cucumis sativus. This was confirmed by ELISA, however in other two species (Cucurbita maxima and Sechium edule), it was negative. Similar observations were made in Luffa acutangula by Kader et al. (1997) by Dot Immuno Binding Assay (DIBA). In Luffa operculata, a natural incidence of PRSV-W was confirmed by Lima et al. (1997) by serological method; they concluded that it acted as a reservoir host for the survival of PRSV. In another survey conducted by Zouba et al. (1997) in Batina region in Oman, 320 commercial cucurbit fields were screened during 1994-95 and 95-96 for the presence of PRSV by ELISA; 716 symptomatic samples collected from squash, watermelon muskmelon, pumpkin and bottlegourds were positive for PRSV.

The major areas of field grown melon were surveyed in Spain for the occurrence of PRSV-W strain and other viruses during 1995 and 1996. Of the 152 plant samples tested by ELISA for the presence of PRSV-W, very few were positive, while the rest were found to be positive to CMV and ZYMV (Luis et al., 1998; Wang et al., 1998). Forty out of 231 samples collected from six different cucurbit crop species - Luffa cylindrica, Citrus lanatus, Cucurbita moschata, Benincasa hispida, Lagenaria siceraria and Cucumis melo from different areas of Taiwan were found positive to PRSV-P by antiserum and 12 were found positive to PRSV-W serological tests. A mixed infection of WMV, ZYMV, CMV and PRSV was detected by ELISA test from Latin province by Roggero et al. (1999) in Cucurbita pepo, during the survey conducted during 1997-98. From Lebanon, Abou et al. (2000) reported heavy
economic losses in commercial cucurbit production by ZYMV followed by WMV and PRSV-W and to a lesser extent by CMV.

Yuki *et al.* (2000) from Brazil surveyed 38 cucurbit species from 40 agricultural regions for the incidence of CMV, PRSV-W, and WMV-2, *Zucchini Lethal Chlorosis Virus* (ZLCV) and ZYMV during 1997-99. Among 621 samples tested by Plate Trapped Antigen (PTA) ELISA, PRSV-W and ZYMV were found in high frequency accounting for 49.1 and 24.8%, respectively, and rest of the viruses were below 10% each. Moura *et al.* (2001) collected 118 leaf samples showing viral symptoms from the field cultivated with *Cucurbita moschata* (46), *Citrullus vulgaris* (30), *Cucumis anguira* (23), *Cucumis sativus* (13) and *Cucumis melo* (6) and were tested by ELISA and DIBA for the presence of viruses. The PRSV was detected in 64.40% of samples followed by WMV-2 (15.40%), CMV (6.80%), SqMV (3.40%) and ZYMV (3.40%). The frequent incidence of PRSV and ZYMV was reported in cucurbits in the mediterranean, sub-tropical and tropical regions by Lecoq *et al.* (2003) and they suspected the possibility of movement of PRSV through melon, in the field.

Walters *et al.* (2003) surveyed the incidence of the most prevalent viral pathogens infecting cucurbit crops during 1998 - 2000 in muskmelon, cucumber, marrow, pumpkin, squash and watermelon in Illinois. They found the incidence of WMV to a tune of 84% and CMV, PRSV, SqMV and ZYMV to 8, 6, 9 and 1%, respectively, in the samples tested.

In recent years, mosaic disease has been one of the major constraints in the cultivation of bottlegourd which is an important vegetable crop of Maharashtra State. Surveys indicated that bottlegourd mosaic disease is endemic to Nashik, Ahmednagar and Pune regions, where the incidence varied from 10-100%. The study by Mantri *et
al. (2004) revealed that the *bottlegourd mosaic isolate* (BgM-NW-3), collected from Wadner, and Nashik produced characteristic symptoms of mosaic, mottling, interveinal chlorotic bands, leaf distortion, malformation of fruits and reduction in fruit size.

**Cucumovirus:** Survey of cucurbits caused by several viruses including members of the Cucumovirus, Comovirus, Tobamovirus, and Potyvirus group were carried out to understand the symptoms (Mukhopadhyay, 1985). Leaves of infected vines of summer squash exhibited typical mottling, vein clearing, marginal chlorosis, blisters or puckering in interveinal areas. Symptoms caused by Cucumovirus in muskmelon includes severe stunting, yellow mosaic pattern of foliage, reduction in the size of leaves and internodes, interveinal chlorosis, followed by bright yellow colour in older leaves. Severely infected leaves became distorted with extensive reduction in the size of leaf lamina; as a result, leaf size is reduced in to a thread-like structure and hence termed “shoe-string” and infected vines produced few small sized and malformed fruits (Cheema et al., 1999).

**Tospovirus:** The genus *Tospovirus* of family *Bunyaviridae* is an enveloped isometric RNA virus with a tripartite genome containing small (S), medium (M) and large (L) segments of ssRNA. They are transmitted by thrips (Order, Thysanoptera) in a propagative manner, and is one of the most important plant virus groups infecting a wide range of economically important crop plants all over the world (Varma et al., 2002; Pappu et al., 2009). Due to the economic impact of the viruses on a wide range crops, most of the research was carried out in India, although tospoviral diseases cause significant problems in several other countries including Bangladesh, Nepal, Pakistan and SriLanka.
Groundnut bud necrosis virus (GBNV) and Watermelon bud necrosis Virus (WBNV) are widely distributed in India, and are endemic to many states including Andhra Pradesh, Gujarat, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, TamilNadu, Uttar Pradesh and West Bengal.

Cultivation of watermelon was seriously affected due to the out break of WBNV in southern India, forcing farmers to abandon growing of watermelon due to total crop loss (Singh and Krishnareddy, 1996). Disease symptoms similar to those induced by Tospoviruses have been described in India since 1960’s in several crops such as black gram (Vigna mungo), brinjal (Solanum melongena), hot pepper (Capsicum annum), cowpea (Vigna unguiculata), groundnut (Arachis hypogea), mungbean (Vigna radiata), pea (Pisum sativus), and potato (Solanum tuberosum) (Nariani et al., 1963; Amin et al., 1979; Narayanaswamy et al., 1975; Ghanekar et al., 1979; Khurana et al., 1989; Krishna Reddy and Varma, 1990; Bhat et al., 2002). Tospovirus was a monotypic genus until 1990. A new and unusual disease in watermelon characterized by leaf mottling and die-back of shoots, was observed during 1991-92 in parts of southern India (Singh and Krishnareddy, 1996), and a distinct Tospovirus, WBNV, was found associated with the disease (Jain et al., 1998).

The most recently reported Tospovirus in India includes Iris yellow spot virus (IYSV) on onion (Alium cepa) (Ravi et al., 2006) and garlic (Alium sativum) (Gawande et al., 2010).

The occurrence of disease caused by WBNV and GBNV was studied on several crop species. In most cases, the natural infection on various host species was initially reported to be caused by TSWV. The molecular characterization revealed that the virus is Groundnut bud necrosis virus (GBNV) which was reported to cause
spotted wilt in tomato (Rao et al., 1980) and pea (Rao et al., 1985), leaf curl in mungbean and urdbean (Amin et al., 1985, Rao et al., 2003), necrosis in cowpea, chilli and brinjal (Rao et al., 1987), stem necrosis in potato (Khurana et al., 1997), bud blight in soybean (Thakur et al., 1998) and rajmah (Akram and Naimuddin, 2009). Ghanekar et al. (1979) indicated that the disease occurred in every groundnut growing regions of India with an incidence ranging from 5-80%. Reddy et al. (1983) reported that the disease was endemic to Andhra Pradesh and Tamil Nadu and observed its wide-spread occurrence in many other regions of Maharashtra, Gujarat, Rajasthan and Uttar Pradesh.

The watermelon bud necrosis incidence was 39-100% with an estimated yield loss of 60-100% (Krishnareddy and Singh, 1993). Surveys conducted in the watermelon growing areas of Karnataka revealed that the incidence of disease varied from 20-100% depending upon the time of infection and cultivar grown (Singh and Krishnareddy, 1995). The overall incidence of Bud necrosis virus disease in Bangalore, Kolar, Chitradurga and Mandya districts of Karnataka ranged between 0.7-10% up to 30-45 days after sowing and later increased to 10-100% up to 60-100 days after sowing (Krupashankar, 1998). Pandey and Pandey (2001) reported a very high (70%) incidence of watermelon bud necrosis disease in the watermelon experimental field at Indian Institute of Vegetable Research, Varanasi, India. In Indian Institute of Horticultural Research, Hesaragatta (IIHR) experimental fields, WBNV infection was not visualized up to three weeks after sowing watermelon, irrespective of the month of sowing, but by fourth week the infection increased to 4-16% in all plantings and later spread to nearly 100% of plants by eleventh week (Krishnakumar et al., 2006). The incidence of bud necrosis in watermelon ranged from 0 to 40% in Maharashtra during 2004 and from 1-30% in Karnataka during
2002. High incidence of bud necrosis disease was observed in Kalegaon area in Jalna district in Maharashtra up to 40% (Bhanupriya, 2006). Surveys carried out on watermelon, chilli, tomato and other horticultural crops in Tumkur, Chitradurga, Raichur, Nellore and Pune, indicated that the incidence of WBNV ranged from 30-80% in watermelon (NS 295) crop sown in July 2006 on-wards at Tumkur. Similarly, in Chitradurga NS 295 variety sown from November on-wards showed WBNV incidence of 50-80%. In Nellore, the low incidence of WBNV (10%) was noticed except in Venkatagiri (30-80%) and in Pune the incidence ranged from 40 to 60% (Anon, 2007).

The genetic diversity in IYSV, CaCV, and WBNV occurring in India has been studied based on fewer isolates as compared to GBNV (Kunkalikar et al., 2011). The diversity in PYSV, however, has not been studied so far. The N gene sequence of 31 IYSV isolates from onion showed a divergence of up to 5.2% at the amino acid level. The WBNV is a serious problem in watermelon, and 12 isolates that were sequenced showed 7.0-8.55% divergence based on amino acid sequences of N and NSm proteins, respectively.

**Tobamovirus:** The host range of the virus includes several plant species of the family cucurbitaceae. The virus designated as *Cucumber fruit mottle mosaic virus* (CFMMV) caused severe mottling or mosaic on cucumber fruits and its spread within the greenhouse could lead to significant economical losses in cucumber crops.

**Begomoviruses:** During 1950-70 period, begomoviral disease in cucurbits was of minor importance in India. During this time, only *pumpkin, (Cucurbits moschata) yellow vein mosaic* (PYVM) disease was known to occur in the central–western India (Verma, 1955). It attracted the attention as emerging disease problem since 1981, when many cucurbits such as bittergourd and winter squash were found to be affected
by Begomovirus disease (Raj and Singh, 1996; Verma and Giri 1998; Singh et al., 2001; Khan et al., 2002). Recently, two different Begomoviruses, *Squash leaf curl China viruses* (SLCCNV) in pumpkin (Muniyappa et al., 2003) were observed in India.

Sohrab et al., (2010) observed 4.7-36% disease incidence of Begomovirus of bottlegourd in the state of Haryana during 2003-06. The severely affected plants were stunted and produced very small chlorotic and mildly curved leaves.

Many different begomoviral infections have been reported from many other countries. For example, *Squash leaf curl Yunnan virus* from China (Xie and Zhou, 2003), *Melon leaf curl chlorotic leaf curl virus* from Guatemala (Brown et al., 2001), *ToLCNDV [Luf]* from Thailand (Sametwanich et al., 2000), *Watermelon chlorotic stunt virus* from Sudan and Iran (Kheyr-Pour et al., 2000), *Squash leaf curl Phillipines virus* from Phillipines (Kon et al., 2003), *Cucurbit leaf curl virus* (Brown et al., 2000), *Cucurbit Leaf crumple virus* (Guzman et al., 2000), *Squash leaf curl virus* (Flock and Mayhew, 1981; Lazarowitz and Ladnis, 1991) and *Squash mild leaf curl virus* (Brown et al., 2002) from USA and *Luffa yellow mosaic virus* from Vietnam (Revil et al., 2003).

**Economic importance**

The GBNV, WBNV, GYSV, IYSV and CaCV are five distinct Tospoviruses that are reported to cause disease in many economically important crops in the Indian sub-continent (Reddy et al., 1992; Singh and Krishna Reddy, 1996; Satyanarayana et al., 1998; Ravi et al., 2005 and Kunkalikar et al., 2007). The yield loss was estimated at 50-100% in groundnut due to GBNV infection (Reddy et al., 1983). Further, the yield loss due to GBNV in India was estimated at more than 80% (Dasgupta et al., 2003). The above diseases resulted in losses through reduction in growth, and yield,
and were responsible for distortion and mottling of fruits making the product unmarketable. More than 25 viruses belonging to genera Cucumo, Como, Tobamo, Poty and Ilarvirus are known to infect cucurbit crops world-wide (Lovisolo, 1980).

*Pumpkin yellow mosaic disease* (PYVMD) causes significant damage to pumpkin production throughout India. An epidemic of PYVMD was documented for the first time in south India in 2004 with disease incidence reaching up to 100% and significant yield loss (Maruthi et al., 2007). This resulted due to a combination of factors including the large number of B-biotype populations, the B-biotype have the ability to transmit the virus efficiently and cultivation of susceptible varieties.

In 1990, a severe epidemic of leaf curl disease in muskmelon and yellow vein mosaic in pumpkin caused by Begomoviruses appeared in northern India (Varma, 1990). This epidemic was caused by an increase in whitefly population in cucurbit in the early growing season. Since then, diseases caused by Begomoviruses have emerged as a major constraint in the production of a variety of cucurbits in northern India (Varma and Giri, 1998). In 2001, over 50% of pumpkin, muskmelon, watermelon and bottlegourd plants were severely affected by Begomoviruses in commercial cucurbit growing areas (Sohrab et al., 2003). Yellow vein mosaic of cucumber, which emerged in the early 1990s in the north-central plains of India, is caused by a bipartite Begomovirus (Raj and Singh, 1996). Spongegourd. Its cultivation in India is adversely affected by the high incidence (>90%) of mosaic disease. The disease is caused by a bipartite Begomovirus, which was provisionally termed *Luffa yellow mosaic virus* (LYMV). The LYMV is easily transmitted by whiteflies to other common cucurbits like bottlegourd, cucumber and muskmelon (Varma and Giri, 1998).
Biological characterization

Symptomatology

Symptoms vary considerably depending on the cucurbit species, cultivar, viral strain and environment factor. Foliar symptoms include green mosaic, rugose leaf, green vein banding, chlorotic rings and malformation. These symptoms are prominent in some winter and summer squashes, but the affected leaves were near normal in size. Fruits were not distorted, but in some winter and summer squashes, leaf development was affected, fruits are distorted, and coloration was adversely affected by green spots, particularly on yellow fruits.

Potyviruses: Dahal et al. (1997) observed severe mosaic, leaf distortions, blisters and shoe-string in squash, while mosaic or yellow mosaic, leaf distortion and blisters in other cucurbits infected by PRSV. Kader et al. (1997) found that samples which showed positive reaction to PRSV-P antisera exhibited mosaic, vein clearing and leaf curl and samples which were positive to PRSV-W showed chlorotic spot and interveinal chlorosis. The other types of symptoms observed were necrotic, severe mottle and mild mottle along with deformation of leaves in PRSV infected cucurbits (Kuan et al., 1999). Similarly, limited necrosis with mottle was observed in Cucumis metuliferus and Cucurbita pepo and systemic symptoms in Melothria pendula and Momordica charantia (Gonzalez et al., 2002). To demonstrate the versatile nature of symptoms, Pacheco et al. (2003) introduced terms like mild strain, which caused no symptoms and severe strains that caused severe mosaic symptom in squash and watermelon. Symptoms due to PRSV also differed in some cucurbits. In a study of the host range of PRSV, chlorotic spots and mottling in Luffa acutangula, mottling, mosaic, puckering along with vein clearing in Cucumis sativus and Cucumis pepo,
and chlorotic and necrotic spots on *Cucumis melo* var. *utilissium* were observed (Singh *et al.*, 2003).

Cultivars of *Cucurbita pepo*, *Cucumis melo* and *Citrullus lanatus* affected by virus incited yellow mosaic, severe malformation, blisters, extreme reduction in the size of lamina, necrosis and severe plant stunting. Squash and pumpkin developed knobby areas, which cause prominent deformation. Melon and watermelon fruits were also malformed that often developed deep longitudinal and radial cracks. Seed production is drastically reduced, and seeds are frequently deformed. Depending upon the strain involved, symptoms strongly resemble those caused by *Papaya ring spot virus* type W (PRSV-W). In tropics, ZYMV is often associated with PRSV-W or with *watermelon mosaic virus* (WMV), and serologically it is related to WMV but not to PRSV-W.

C Kapoor and Verma (1958) reported that symptoms of faint chlorotic spots appeared on inoculated leaves within 6-8 days, followed by premature dropping and wilting. After sap inoculation of PRSV, a variety of symptoms on leaf viz., vein clearing, mild mosaic, downward curling of leaf, malformation and thickening of vein-lets were recorded in Uttar Pradesh (Singh *et al.*, 2003).

Symptoms caused by *Squash Mosaic Virus* (SqMV) are variable, depending on the host species and cultivar. Generally, the infected plant responds with a variety of symptoms, including green vein banding, mosaic, mottle, blister, ring-spot, and protrusion of veins at the leaf margin. Under certain environmental conditions, infected plants of some squashes (*C. pepo* and *C. moschata*) may develop prominent foliar enations and plants show stunted growth with malformed and mottled fruits.

Early symptoms of virus infestation consist of vein clearing and crumpling of cucumber young leaves. More mature leaves may be decolorized. Depending upon the
virus strain, symptoms vary from mild to severe leaf distortion, light and dark green mottling, yellow or silver leaf flecks and plant stunting. Fruits rarely have symptoms, but with strains they can become more severely distorted or develop chlorotic or silver spots and streaks, especially at high temperature. All cucurbits are susceptible to cucumber mosaic virus, although it rarely affected watermelon. Symptoms first appear on younger leaves which curl downward and become mottled, distorted, wrinkled and reduced in size.

**Cucumoviruses:** Survey of cucurbits affected by several viral pathogens of Cucumovirus, Comovirus, Tobamovirus, and Potyvirus groups was carried out by Mukhopadhayay (1985). Leaves of infected vines of summer squash exhibit typical mottling, vein clearing, marginal chlorosis, blisters or puckering on interveinal areas. Symptoms caused by Cucumovirus in muskmelon include severe stunting, yellow mosaic pattern of foliage, reduction in size of leaves and internodes, interveinal chlorosis, followed by bright yellow colour on older leaves. Severely infected leaves were distorted with extensive reduction in the size of leaf lamina, and as a result, leaf size is usually reduced in to a thread like structure shoe-string and infected vines bear a few small sized and malformed fruits (Cheema *et al.*, 1999).

**Tobamoviruses:** Cucumber green mottle mosaic virus is found in naturally infected cucumber, watermelon, and melon crops, but apparently not in vegetable marrow (*Cucurbita pepo*). It occurs also in bottlegourd (*Lageneria siceraria*) which is used as root-stock for watermelon cultivation. In case of *Cucumis sativus*, symptoms were the absence of local lesions, systemic slight vein clearing, and after two weeks of infection, light and dark green mottle with leaf puckering, and blistering and leaf malformation especially in winter. Infected plants are stunted (Hoolings *et al.*, 1975).
**Tospovirus**: Symptoms caused by Tospovirus species are highly variable and of little diagnostic value. A wide variety of symptoms were observed in Tospovirus infected plants. Systemic symptoms include necrosis leading to partial or complete plant death, wilting, fruit abortion, stunting, leaf deformation, mosaic, chlorosis, and mottling and ring formation, depending on the host plant, season and environment (German et al., 1992).

The field symptoms of WBNV in watermelon initially develop as chlorotic mottling, yellow spots or patches, and mild crinkling of leaves. Subsequently, necrosis of buds in the growing tips result in die-back of vines. In the young crop, rapid die-back and wilting of plants caused a total loss of the affected plants. In mature crop, shortened internodes, upright growth of younger shoots, necrosis on stem, petiole, and fruit stalk are commonly seen. Infected plants produce unmarketable small, deformed fruits with uneven surface, and necrotic or chlorotic rings, depending on the cultivar. The GBNV is predominant in leguminous and solanaceous hosts, while WBNV is largely confined to curbitaceous hosts such as ridge gourd and cucumber (Mandal et al., 2003; Jain et al., 2007).

Krishnareddy and Singh (1993) observed an unusual disease symptom in watermelon characterized by leaf mottling, yellowing and necrotic streaks on veins, shortened internodes, necrosis and die-back of buds. Symptoms of watermelon bud necrosis disease occurring in India include necrosis of buds and petioles, necrotic spot on leaves and necrotic streak on vines. The vines eventually wilted and dried, and fruits from infected plants of few varieties exhibited broken concentric rings with corky texture on the surface (Singh and Krishnareddy, 1996).

Krupashankar (1998) observed the bud necrosis disease symptom on all plant parts above the ground. The foliar symptoms include mild mottling, crinkling,
yellowing and dark-brown or black coloured necrotic spots, rugosity of young leaves and narrowing of leaf lamina. Necrosis on the midrib of leaves was one of the major symptoms of the disease. Mid and all lateral veins turned black, became thick and at times, distorted. Severe disease symptoms were observed in the young tender branches. The affected plants were severely stunted, had shortened internodes and became very brittle. The upright growth of younger branches, non-opening of flower buds, bud necrosis, and die-back were the major symptoms of this disease. Another conspicuous symptom was the presence of longitudinal brown necrotic streaks on vine tendrils, petioles, and fruit stalks. As the disease progressed, the stem exhibited splits and started drying from the tip. Fruit set and yields were drastically reduced, fruits of a few varieties showed necrotic or chlorotic ring spots with corky texture. Some fruits showed chlorotic mottle symptoms and uneven surface. Fruit size, shape and quality were affected resulting in poor market value of fruits (Bhanupriya, 2006).

**Begomoviruses:** Sohrab *et al.* (2003) studied the mosaic disease of sponge gourd. Symptoms of disease yellow spots occurred on emerged leaves, and misshapen fruits. Nearly 100% of sponge gourd plants were symptomatic in Delhi. Khan *et al.* (2002) observed severe disease in bitter melon with virus like symptoms at Lucknow and symptoms consisted of upward curling, shortening and distortion of leaves. Singh *et al.* (2001) studied the yellow mosaic in *Cucurbita maxima* in and around Lucknow, India and symptoms of yellow mosaic disease were reproduced in seedlings. Mizutani *et al.* (2011) studied the yellow green mosaic symptoms on leaves in Kiaten, Central Java, Indonesia. The disease caused by Begomovirus symptoms severe leaf curl. It was associated with *Squash Leaf curl China virus*.

Tiwari *et al.* (2012) conducted a survey during 2007-2008 in Uttar Pradesh. The typical symptoms of Begomovirus (Yellow mosaic and Yellow vein) were
observed on six cucurbitaceous crops *viz.*, bittergourd, pointedgourd, squashes, pumpkin, spongegourd and ridgegourd cultivated in North India. The presence of Begomovirus was detected from the total DNA extracted from the susceptible infected leaf samples of these species by PCR using specific primers of well characterized Begomoviruses.

The SLCV infected bean and squashes, while WCMoV infected bean, cucumber, melon, squash, watermelon and tobacco. In melon and cucumber, SLCV caused characteristic leaf curling, and sometimes together with a mild mosaic. A bright yellow mosaic or mottle accompanied by leaf curling or enations on the undersides of leaves and severe stunting occurred in all susceptible species of *Cucurbita* and in watermelon. The infected squash plants were severely stunted and failed to produce additional foliage after the infection. Infected cucurbits often produced abundant flowers with either delayed opening or shed they were soon after blooming, and rarely set fruits. Fruits that set rarely matured and fruits set immediately prior to infection were often misshapen, discolored and slightly bumpy, and were usually not of marketable quality. In watermelon, early infection resulted in a foliar mottle of leaves on shortened runners, with runners often growing or curling upwards, off the ground. Fruit set early in the season, prior to infection were marketable (Brown and Nelson, 1989).

**Host range**

The CMV is world-wide in distribution and could cause infection in about 800 plant species, including both monocots and dicots. The host range included many well known vegetable crops (carrot, celery, cucurbits, legumes, lettuce, onion, pepper, spinach, and tomato) and ornamentals (anemone, aster, delphinium, geranium, lily, periwinkle, petunia, primula, viola, and zinnia). The host range of the virus was tested
by using *Myzus persicae* as the vector. Results indicated that out of 53 species of plants tested, the virus infected only certain members of cucurbitaceae and no symptomless carriers were noticed.

*Cucumber Green Mottle Mosaic Virus* (CGMMV): is commonly found in areas of northern Europe, the Netherlands, UK, or Japan, where protected crops were grown. Strains of the virus were reported in watermelon in Japan and melons and bottlegourd in India and Iran. The biological and molecular techniques were employed to determine the new cucurbit-infecting Tobamovirus. The uncharacterized virus was isolated from the greenhouse cucumber plants. The biological and serological data indicated that the virus belonged to genus Tobamovirus.

Tospoviruses have a very broad host range that spans more than 1,100 different species and more than 80 families within, which include both monocots as well as dicots under experimental conditions. Susceptibility to infection by Tospoviruses is still found in many plant families. From the view point of agricultural importance, the susceptible host species were found in many families (Peters, 2003). Many of these plants are hosts for both viruses and thrips and they serve as reservoirs of infection that contribute to epidemics on crop plants (Cho *et al.*, 1986). Survey conducted to understand the distribution of Tospoviral pathogens such as *Peanut bud necrosis virus* (PBNV), *Watermelon bud necrosis virus* (WBNV), *Capsicum Chlorosis virus* (CaCV) and *Irish Yellow spot virus* (IYSV) during 2002-09 in the major vegetable growing areas in India indicated that PBNV was documented widely in tomato and chilli peppers in 14 states representing the southern, northern, western, north eastern and central regions and WBNV was predominantly detected in watermelon and cucurbits.
Among 37 species, WSMoV infected 23 plant species representing six out of nine families tested. The virus systemically infected many species of cucurbitaceae and solanaceae (Iwaki et al., 1984). The WSMoV (from Taiwan) and TSWV-NY (tomato isolate of New York) caused similar symptoms in test plants of families amaranthaceae, chenopodiaceae, and solanaceae (Yeh et al., 1992). With the exception of WSMV from Japan (Iwaki et al., 1984) and watermelon isolate of TSWV from Taiwan (Yeh et al., 1992), most of the Tospoviruses caused only local infections in cucurbitaceous crops (Je, 1970; Reddy and Wightman, 1988).

Singh and Krishnareddy (1996) showed that the watermelon tospovirus isolate in India systemically infected watermelon, muskmelon and other cucurbits. They evaluated 33 plant species belonging to seven families in the host range studies of WBNV. Among them, only 17 produced different types of symptom; only necrotic local lesions were observed in *Chenopodium amaranticolor*, *Petunia hybrid*, *Solanum melongena*, *Lycopersicon esculentum* cv. Arka Sourabh, and only chlorotic local lesions were observed in *Vigna unguiculata* cv. C-152, *Vigna mungo* and *Sesamum indicum* whereas, chlorotic or necrotic spots followed by systemic infection were observed on *Citrullus lanatus* cv. Arka Manik, *C. lanatus*, cv. Madhu, *Cucumis melo*, *Cucumis sativus*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo*, *Lagenaria siceraria*, *Trichosanthes anguina*, *Arachis hypogaea* cv JL-24, *Cassia tora*, *Lablab purpureus*, *Glycine max* cv. Bragg, *Phaseolus lanatus*, *P. vulgaris* cv. Arka komal, *Capsicum annuum* cv. Yellow Wax, *Datura stramonium* *Lycopersicon esculentum* cv. Pusa Ruby, *L. esculentum* cv. Arka Sourabh, *Nicotiana glutinosa*, *N. rustica*, *N. tobacum* cv. Samsun, *Physalis floridana*, *Gomphrena globosa* and *Emilia sonchifolia*.

Krishnaveeni et al. (2004) tested 31 plant species by mechanical inoculation with an objective of studying the host range for six different GBNV isolates collected
from different crops species viz., tomato, groundnut, black gram, peas, chilli and brinjal. Out of them, visible symptoms were produced by all six isolates on only nine plant species viz., lima bean, green gram, soybean, black gram, groundnut, tomato, brinjal, chilli and tobacco. However, irrespective of the isolate inoculated, 20 plant species showed positive reaction to GBNV by back inoculation. None of the isolates produced symptoms or showed positive reaction upon inoculation to hosts such as beet root, sunflower, safflower, cabbage, snake gourd, ash gourd, bottle gourd, okra and potato.

Krupashankar (1998) recorded the difference in type of symptoms that are observed upon mechanical sap inoculation of Tospovirus on ornamental and vegetable crop plants belonging to several families. *Gomphrena globosa* showed necrotic local lesions on leaves. Chlorotic local lesions turning to necrotic lesions were observed in *Chenopodium amaranticolor* and *Chenopodium quinoa*. Watermelon plants (*Citrullus lanatus* cv. Arka Manik and cv. Madhu) showed chlorotic local lesions followed by necrotic local lesions and systemic infection. The other cucurbitaceous plants like *C. sativus* and *C. melo* showed mild chlorotic spots on leaves. Similar spots were observed in *Glycine max* cv. Bragg, *Vigna mugo* and *Capsicum annum* cv. Yellow Wax. Chlorotic spots followed by necrotic local lesions were observed in *Datura stramonium*, *Lycopersicum esculentum* cv. Pusa Ruby, and cv. Arka Sourabh and *Physalis floridana*. Whereas, *A. hypogaea* cv. JL-24 produced chlorotic local lesions and crinkling of leaves. *Vigna unguiculata* cv. C-152 and *N. rustica* produced conspicuous chlorotic spots followed by necrotic local lesions on their leaves. *N. benthamiana* showed systemic symptoms like paleness and dwarfing of leaves, while plants of *N. glutinosa* expressed mild chlorotic local lesions on leaves.
By mechanical transmission, WBNV-Wm JLN isolate was inoculated on 21 host plants belonging to six families, out of which 13 hosts were successfully infected. Out of the 13, eight hosts exhibited local chlorotic or necrotic lesions, while four hosts viz., *N. rustica* *N. glutinosa*, *D. stramonium* and *C. lanatus* showed systemic infection. *Vigna unguiculata* cv. C-152, *N. tabacum* cv. Xanthi, White burley and Samsun, and *N. benthamiana* produced chlorotic lesions in 6 to 8 dpi. *Chenopodium guinoa*, *C. amaranticolor*, and *Petunia hybrida* produced local chlorotic lesions followed by necrotic lesions on the inoculated leaves. *Gomphrena globosa* produced local necrotic lesions; *N. rustica*, and *N. glutinosa* produced local chlorotic or necrotic lesions followed by wilting of plants. *Datura stramonium* and *Vinca rosea* produced local chlorotic and necrotic lesions on the inoculated leaves, followed by systemic infection with mosaic mottling and finally wilting of the plant (Bhanupriya, 2006).

Khan *et al.* (2002) studied the association of Begomovirus with bitter melon in India, where monopartite Begomovirus caused yellow mosaic disease of pumpkin (*C. maxima*) in and around Lucknow. It showed the symptom of yellow mosaic in pumpkin, and in case of cucumber, of Begomovirus was associated with the yellow mosaic symptoms (Raj and Singh, 1996).

Brown *et al.* (2002) observed the incidence of CuLCV from the symptomatic cucumber (*Cucumis pepo*), honey dew melon, muskmelon (*Cucumis melo*), pumpkin (*Cucurbita maxima*) and squash (*Cucumis pepo*) in Arizona, Texas, USA.

**Vector transmission**

**Transmission by aphids**: Viral pathogens affecting cucurbits include cucumber mosaic virus (CMV), *Papaya ring spot virus* pathotype (PRSV-W), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). All the above viral pathogens are spread by one or more number of the following aphid species; *Aphis*
craccivora, A. glycine, A. gossyphi, A. fabe, A. medicagins, Myzus persicae and others. All the above viruses are transmitted in the non-persistent manner (style-borne) with varying degree of efficiency. The viral pathogen are generally acquired by aphid instars within one minute, but the aphids' ability to transmit them declines rapidly and is lost within a few hours. They can be easily transmitted mechanically, and the use of phosphate buffer facilitates infectivity. The viral pathogens CMV, PRSV-W, WMV, and ZYMV are widespread in the temperate as well as warm regions of the world, where they often cause severe loss.

Lecoq et al. (1992) reported that aphids act as a major vector in transmitting CMV, PRSV and Tobacco ring spot Nepo Virus in melon plants. Similarly, the transmission of CMV, PRSV and ZYMV was confirmed in summer squash by aphids (Brown et al., 1993). The non-persistent nature of transmission of PRSV-W and Potato Leaf Roll Virus (PLRV) was confirmed by Diaz et al. (1993). They also showed that the use of acetone is the best means of collection of aphids for their transmission assay. An attempt to transmit of PRSV-W and ZYMV pathogens by six aphid species was carried out by Chao et al. (1994), out of which Aphis gossypii and Myzus persicae were found to be predominant by involved in transmitting both viral pathogens. The efficiency of four aphids in the transmission of two mild strains of PRSV-W was studied. All the four species viz., Aphis gossypii, Lipaphis erysmi, Myzus persicae, and Toxoptera citricidus were capable of transmitting the pathogen to Cucurbita pepo and the latter aphid was found to be the most efficient (Giampian and Rezende, 2001). Reddy (2000) reported that M. persicae, A. gossypii and Aphis craccivora transmitted PRSV from papaya to papaya by 90%, 70%, and 50% respectively. Maximum transmission of 90% was recorded with 12 aphids per plant and minimum of 10% with one aphid.
Transmission by thrips

*Watermelon silvermottle virus* (WSMoV) and *Watermelon bud necrosis virus* (WBNV) are transmitted by thrips that infect some cucurbits. Like most other plant viruses, Tospoviruses are vectored by arthropods for their spread in host plant population. The TSWV is transmitted in nature by seven species of thrips: *Frankliniella fusca*, *F. intonsa*, *F. occidentalis*, *F. schultzei*, *Thrips palmi*, *T. setosus* and *T. tabaci*, which are minute insects of the family Thripidae (Moyer, 2000); *F. occidentalis* (the Western flower thrips), *F. schultzei* (the cotton bud thrips), and *Thrips tabaci* (the onion thrips) are the most frequently reported vector species.

Tospoviruses were shown to be transmitted from plant to plant by thrips (Pittman, 1927). The infective adults were capable of transmitting the virus, while the acquisition occurred during the larval stage of the insect (Linford, 1932). A special characteristic of Tospovirus transmission is that the virus can only be acquired by larvae while feeding on infected plants, while the adults cannot acquire the virus (Sakimura, 1963; Ullman *et al.*, 1992). Therefore, the initiation of the infection cycle can occur only when female adult thrips lay eggs on TSWV-infected leaves that are suitable for egg and larval development. The larvae may acquire the virus within a period of 10 minutes or less, though the chance that they will become infected increases with the length of the acquisition period. Approximately, 80% of thrips that transmit the virus did so when they were still larvae. In India, Singh and Krishnareddy (1995) reported *Thrips flavus* as a new vector of Tospovirus infecting watermelon plants. Most of these insects resumed transmission after emergence as adults. The median latent period for the infecting larvae ranged from 80 to 170 h when they were kept at either 20 or 27°C, respectively. This period was similar in length for TSWV and INSV (Wijkamp *et al.*, 1993).
Palmer et al. (1990) and Vijayalakshmi (1994) discovered the occurrence of *T. palmi* in groundnut in India and proved the involvement of the insect in transmission of GBNV. Singh and Reddy (1996) demonstrated the ratings of WBNV transmission as 6/10 (watermelon to watermelon), 3/8 (tobacco) and 3/10 (tomato) within 15 to 25 days after inoculation. Krupashankar (1998) showed 30% transmission of WBNV among watermelon plants through *T. palmi* and it took 30-40 days for the expression of symptoms. Singh et al. (2006) found that potato stem necrosis disease (PSND) caused by *Groundnut bud necrosis virus* (GBNV) is a threat to early stages of potato crop. The virus is not tuberborne but is readily transmitted by thrips, mainly *T. palmi* and inefficiently by *Scirtothrips* spp. but not *Megalurothrips distalis* and *T. hawaiiensis*, (based on bioassay confirmation); most of them were found to be viruliferous mainly *T. palmi*.

**Transmission by whitefly:** Gemini viruses have emerged as serious pathogens of cucurbit crops. During 1950-70s, Begomovirus disease in cucurbits was of minor importance in India. During this time, only Pumpkin yellow vein mosaic (PYVM) disease was known to occur in central-western India (Verma, 1955). This attracted the attention as emerging disease problem since 1981, when many other cucurbits such as bittergourd, cucumber, muskmelon, and spongegourd, and winter squash were found to be affected by Begomovirus disease (Raj and Singh, 1996: Verma and Giri, 1998). Recently, two different Begomoviruses, *Squash leaf curl china virus* (SLCCNV) in pumpkin (Muniyappa et al., 2003) and *Tomato Leafcurl New Delhi-[Luffa]* in spongegourd (Sohrab et al., 2003) have been identified as serious pathogens of cucurbits in India.
Seed transmission

The CGMMV is not transmitted by insect vectors. Infection occurs through roots; when soil recycled substrate contain infected plant debris. It can also be transmitted by contaminated irrigation water or nutrient solutions. The virus is very stable and mechanically transmissible during pruning and harvesting, or simply by rubbing leaves against the infected leaves of neighboring plants. Thus, the effective and most serious form of transmission is by seeds and the rate drops rapidly to one percent, a few months after storage. Secondary spread of the virus is rapid by mechanical transmission during cultural operations. The danger of the virus spreading by means of contaminated irrigation water or plant debris can not be ignored. Apparently seed transmission of viruses is noticed, but in many of the cases it fails.

The pathogen PRSV is demonstrated to be not seed transmitted through papaya seeds (Wang, 1982; Prasad and Sarkar, 1989). However, there was a rare incidence of two out of 1355 seedlings, showing symptoms closely related to PRSV (Bayot et al., 1990). In contrast to the above, Shaikh (1996) in his experiment involving one thousand seedlings raised from seeds of PRSV infected papaya fruit, even after 12 months, did not obtain infected seedlings. Similar observations were recorded by Reddy (2000).

Reddy et al. (1983) reported that early GBNV infection in groundnut produced shriveled seeds of which only 30% germinated; however, the virus was not detected in seedlings raised from the infected plants. Infective viruses were recovered from the test a of immature and freshly harvested mature seeds, but the virus was not serologically detected in the test of dried seeds. Neither serologically detectable nor infective virus was recovered from cotyledons and embryos of freshly harvested or
dried seeds. Freshly harvested seeds with test a containing virus failed to transmit the virus. As of now, there are no reports on seed transmission of Tospovirus.

**Epidemiology**

With the presence of wide host range and many weeds, cultivated crops are reservoirs of Potyvirus and Cucumovirus pathogens. Non-persistent transmission of the virus by more than 60 aphid species, including *Myzus persicae* (Sulzer), * Macrosiphum euphorbiae* (Thomas), and *Aulacorthum solani* (Kalthenbach) has been reported. Generally, the virus is acquired by aphid instars within 1 min; their ability to transmit virus declines with in 2 hours. However, the transmission depends on several factors, such as vector biotype, virus strain, environmental conditions, and the season. The CMV is easily transmitted mechanically, and although there is no evidence that it is seedborne in cucurbits, it can be carried in seeds of 19 other plant species (Doolittle, 1920).

During the survey of commercial fields of bottlegourd, plants exhibited severe mosaic, inter-veinal chlorosis and leaf deformation that resulted in the fern-leaf appearance and fruit distortion in approximately 70% of plants. The virus isolate was identified as *Zucchini yellow mosaic virus* (ZYMV) by biological and serological techniques. (Verma et al., 2006).

Cucurbits growing area in different agro-climatic zones of Punjab were surveyed during 2002-03 for various types of mosaic diseases in cucurbits. Based on the symptom expression on the indicator plants, host range, transmission studies, virion morphology, biophysical properties, serology, and electron microscopic studies, the isolates were identified as *Cucumber mosaic virus* (CMV) and *Watermelon mosaic virus-1* (WMV-1). (Sandhu and Kangu, 2007).
Since tospoviruses are not seedborne, it is assumed that the primary spread of tospoviruses is by thrips coming from other crops or weeds, whereas secondary spread occurs from infected plants within a field. Bud necrosis disease of groundnut is mostly monocyclic type, and disease incidence depends on the infection by viruliferous thrips that acquire the virus from other crops or alternate hosts. *Ageratum conyzoides* has been shown to support GBNV and vector multiplication (Reddy, 1998). In another study, the spread of WBNV was influenced by the thrips population, maximum temperature and relative humidity (during morning hours). While minimum temperature negatively influenced WBNV incidence, wind velocity and rainfall failed to influence the WBNV spread or thrips population build-up. The rate of spread of WBNV was highest in watermelon cultivars Arka Manik, followed by NS 295 and Madhu Bala (Krishnareddy *et al.*, 2007; Krishnakumar *et al.*, 2006).

Usually, raising melons early (October to February) in the season is advocated in southern India to minimize WBNV, as it coincides with the occurrence of fewer thrips. The WBNV can be devastating even during monsoon (July to October) if there is a dry spell of 25 to 30 days. Thrips need 10 to 12 days from egg to adult development, and a dry spell in the midst of monsoon can facilitate large-scale multiplication and subsequent migration leading to a WBNV outbreak.

**Genomic organization**

The CMV genome consists of three single-stranded ribonucleic acid (RNA) molecules housed in three separate protein capsids. With uranyl acetate-negative staining for electron microscopy, each capsid appears identical and icosahedral in shape, with what appears to be a hollow center. The particles regularly appear to be flattened and distorted (Tolin, 1977). The nucleoprotein capsids, approximately 28-31 nm in diameter, are made up of 180 identical protein subunits in a $T = 3$ symmetry
consistent with the pentamer-hexamer subunit clustering (Smith et al., 2000). The molecular weight of each protein subunit is between 24-25 kDa, depending on the strain.

Tospoviruses have a single-stranded, tripartite RNA genome with segments designated as L, M, and S in the order of decreasing size. The termini of each of the RNA segments consist of an eight nucleotide sequence (5'AGAGCAAU 3') that is strictly conserved. The remaining un-translated region at the termini also has a high degree of complementarities. Base pairing at the termini between the inverted complementary sequences support a pan-handle structure that most likely serves as a promoter for replication. The L RNA is 8.9 Kb and codes for the L or RdRp protein in the viral complementary (vc) sense. The M and S RNAs are in ambisense orientation. The M RNA is 4.8 Kb and codes in the viral sense for the non-structural protein NSm and for the GN/GC precursor glycoprotein in the vc sense. The S RNA is 2.9 Kb and codes in the viral sense for the nonstructural protein NSs and the nucleocapsid protein in the vc sense (Tsompana and Moyer, 2008). This genus was distinguished from other genera in the Bunyaviridae by the presence of two ambisense RNA segments (Moyer et al., 1999).

The genome of GBNV was only one characterized and sequenced completely among the reported tospoviruses from India. Genome organization was typical of Tospovirus genome consisting of L RNA (8911 nt), M RNA (4801nt) and S RNA (3057 nt) (Gowda et al., 1998; Satyanarayana et al., 1996a, 1996b). The ambisense coding strategy of GBNV S RNA was confirmed by northern blot analysis using strand specific riboprobes, viral sense strand NP gene and viral complementary sense strand of NSs gene of S RNA. The two ORFs in S RNA were separated by a 773 nucleotide A+U rich inter-genic region (Satyanarayana et al., 1996a).
The presence of sub-genomic RNAs was reported from TSWV in the year 1990 (de Haan et al., 1990). Later, Satyanarayana et al. (1996a) also reported a similar presence of sub-genomic RNA species of approximately 1.4 and 1.65 Kb by studying GBNV infected samples probed with viral sense strand of NP and viral complementary sense strand of NSs genes, respectively.

Kumar et al. (2009) sequenced the Watermelon bud necrosis virus (WBNV) medium (M)-RNA. The M-RNA of WBNV-Del consists of 4,796 nucleotides in length and contained two open reading frames (ORF) in ambisense orientation separated by A-U rich intergenic region (IGR) of 420 nucleotides (nt). The 5' and 3' ends of the M RNA were 55 and 47 nucleotides in length, respectively. The small ORF is in viral sense containing 923 nt encoding a putative movement protein of 307 amino acids (34.22 kDa). The large ORF is in viral complementary sense containing 3360 nt encoding a precursor of glycoproteins G1 and G2 of 1119 amino acids (124.44 kDa).

Whitefly transmitted geminiviruses belongs to the family Geminiviridae are characterized by the geminate (paired) shape particles of 30×20 nm size. The paired particle encapsidated circular single stranded DNA genome of 2,500-3,000 nucleotide length. Members of this family are divided into four genera based on two host range, insect vector and genome organization. The genus Begomovirus (earlier called as subgroup III geminiviruses) comprises whitefly transmitted geminiviruses that infect dicotyledonous hosts and derives its name from the type member, Bean golden mosaic virus, a virus that infects bean in Central America. Members of the Begomovirus are transmitted by only one species of whitefly, B. tabaci, and cause severe leaf curl, yellow mosaic and yellow vein mosaic diseases in several important crops and weeds.
<table>
<thead>
<tr>
<th>Open reading Frame (ORF)</th>
<th>Putative protein</th>
<th>Predicted molecular weight (k Da)</th>
<th>Predicted function</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV2</td>
<td>Pre-coat protein Movement Protein (PCP)</td>
<td>~ 13.8</td>
<td>Movement in monopartite</td>
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<tr>
<td>AV1</td>
<td>Coat protein – CP</td>
<td>~ 29.8</td>
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<tr>
<td>AC1</td>
<td>Replication initiation Protein – Rep</td>
<td>~ 40.7</td>
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<tr>
<td>AC2</td>
<td>Transcription Activator protein - TrAP</td>
<td>~ 17.0</td>
<td>Transcription activation of rightward ORFs</td>
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<tr>
<td>AC3</td>
<td>Replication enhancer</td>
<td>~ 15.6</td>
<td>Replication enhancement</td>
</tr>
<tr>
<td>BV1</td>
<td>Nuclear shuttle Protein – NSP</td>
<td>~ 29.2</td>
<td>Nuclear import/ export</td>
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<tr>
<td>BC1</td>
<td>Movement protein – MP</td>
<td>~ 32.4</td>
<td>Movement across Plasmodesmata</td>
</tr>
</tbody>
</table>

(Source: Kings et al., 2011)

Coding region: In DNA A, viral sense strand has two open reading frames (ORF AV2, pre-coat protein and coat protein CP ORF-AV1). In complementary sense, there are two ORFs (ORFAC1 and AC3) encoding, replication initiation protein (Rep) and replication enhancer (REn) protein (Table 3). One more important ORF is ORF AC2, which activates the transcription of rightward ORFs of both DNA A and B (AV1 and BV1) and so called as transcription activator protein, TrAP. In DNA, there is one ORF in viral sense strand coding for nuclear –shuttle protein (BV1, NSP) and one in complementary sense coding for movement protein (BC1, MP) (Table 3).

Intergenic/Common region: Between starting codons of the leftward and rightward coding regions is present a non coding intergenic region. Within the intergenic region is a short stretch of 118-200 nucleotide length segment which is near identical between DNA A and DNA B components. This is the only region, which is near identical in sequence between DNA A and B components and so is called the common region (CR). The nucleotide sequence of CR is highly specific for a given
Begomovirus; it contains important regulatory sequences. The most important elements are, 1. A hairpin or stem and loop structure, where in the loop has the nine nucleotide (nanonucleotide sequence), TAATATTAC which is conserved in all Geminiviruses. It is within the sequence that the rolling circle replication is initiated. 2. There are repeats of 13 bp sequences present upstream of the hairpin structure. The repeat element contains two, 5bp direct repeats separated by central core of 3 bp (GGTAGTAAGGTAG). These repeat sequences are called iterons and they represent the Rep binding sites. The number of repeats and the way they are arranged in the CR is specific for lineage of virus. 3. The segment from the tandem repeat of iteron to end of the stem loop sequences is considered to represent origin of replication (OR1), 4. The promoter of both leftward and rightward genes, TATA box sequences recognized by various transcription factors are also present in the CR. From the genome organization, it is evident, how the function is divided between the two components. Thus DNA A can autonomously replicated in the cell and can get encapsidated, but is dependant on DNA B for movement from cell to cell. DNA B is dependant on DNA A for replication and encapsidation. Since geminate particles contain one molecule of either DNA A or DNA B have to be introduced together by whitefly (Malathi et al., 2004).

Interrelationship between the virus isolates

A survey was undertaken to study the cucurbit crops grown in North-West frontiers of Pakistan during the summer and winter. The survey indicated that plants having mosaic, mottling, chlorosis, and leaf distortion symptoms were frequently found in most of the cucurbit fields. Using the dot immunobinding assay, Cucumber green mottle mosaic virus (CGMMV), Zucchini yellow mosaic virus (ZYMV), Watermelon Mosaic Virus (WMV) and Papaya Ring Spot virus (PRSV) were found
infecting cucurbits. The nucleotide sequences of the coat protein (CP) genes of these four viruses were determined and the deduced amino acid sequence comparisons revealed 88.3-99% similarity of the ZYMV- Pak isolate with other isolates of ZYMV reported world-wide. The amino acid sequence identity of Pakistan isolates of WMV, CGMMV and PRSV ranged from 96.8 to 98.4; 98.1 to 99.4 and 79.3 to 84.2%, respectively. Little variability was observed in the sequence of WMV and CGMMV, and ZYMV-Pak isolate was closer to Indian isolates of the PRSV possibly reflecting the geographical relationship between these isolates (Ali et al., 2004).

Mandal et al. (2003) for the first time reported a natural infection of ridge gourd by WBNV based on infectivity assay, serology and N gene sequence. The comparative sequence analysis of 291bp region of the N gene revealed that the genus Tospovirus infecting ridge gourd shared the highest identity both at the nucleotide (94%) and amino acid (97%) levels with the corresponding region of WBNV, which is a distinct species of WSMoV Serogroup infecting watermelons in India. In contrast, only 76-81 and 82% identity at the nucleotide and amino acid levels, respectively, was observed with the corresponding region of the N genes of GBNV and WSMoV.

The NSm gene is shown to be highly conserved among Indian GBNV isolates as per the study conducted by Akram et al. (2010), who compared the movement protein gene (NSm) of GBNV isolates from cowpea, tomato, groundnut and potato with the corresponding gene from the GBNV type isolate and other tospoviruses. The NP gene sequence analysis from 36 isolates of GBNV collected from nine different crops revealed 94 to 99% identity at nucleotide and 94.9 to 100% identity at amino acid level when compared to GBNV isolate from groundnut (U27809). Similarly other GBNV NP gene sequences from NCBI Gene Bank showed similar amino acid substitution (Bhanupriya, 2006).
The NP genes were amplified ~830 bp fragment from the symptomatic leaves of watermelon bud necrosis isolates. The cloned NP gene nucleotide sequence analysis shared 92% and 84% homology with the known WBNV and PBNV sequences, respectively. The sequence variation at the amino acid level within GBNV (up to 7%) and WBNV (up to 5%) isolates did not correlate well either with the geographical location or host. Both GBNV and WBNV isolates originating from different hosts and locations thus appeared to be a single mixed population with some sub-population, suggesting that there was mixing and movement of isolates (Bhanupriya, 2006). The GBNV isolates were shown to have a wide host range and were commonly found on solanaceous and fabaceous hosts, while WBNV isolates were restricted to cucurbitaceous hosts (Jain et al., 2007).

**Phylogenetic analysis**

During the study on Watermelon bud necrosis tospovirus was found to be a distinct virus species belonging to Serogroup-IV. In this study, the nucleocapsid protein gene of a Tospovirus infecting watermelon in India was cloned and sequenced. The sequence analyses showed that the genes are most closely related to those of *Watermelon silver mottle mosaic mottle* Tospovirus (WSMV) from Taiwan and *peanut bud necrosis* Tospovirus (PBNV) from India, the two definitive species of serogroup-IV, amino acid sequence similarity was 84% and 82% with WSMV and PBNV, respectively. On the basis of the sequence divergence and the previously determined host range differences, the watermelon tospovirus, designated as *watermelon bud necrosis* tospovirus, should be considered as a distinct species belonging to Serogroup-IV (Jain et al., 1998).

Study in Thailand on complete nucleotide sequence of a new isolate of tomato leaf curl New Delhi virus infecting cucumber, bottlegourd and muskmelon, showed
that cucumber virus is an isolate of ToLCNDV, designated as ToLCNDV-[Cucumber: Thailand]. It was isolated and cloned from Begomovirus from bottle gourd and muskmelon exhibiting yellow leaf symptoms. The phylogenetic tree based on the comparison of the complete nucleotide sequence of DNA-A showed that bottle gourd and muskmelon viruses clustered within ToLCNDV-[Cuc; Thai]. The complete nucleotide sequence of DNA-A of bottle gourd and muskmelon viruses have high nucleotide sequence identity (~99%) with that of ToLCNDV-[Cu; Tha]. The nucleotide sequence of the stem loop structure of bottle gourd and muskmelon viruses were identical to that of ToLCNDV [Cuc: tha] which is also associated with yellow leaf disease of bottle gourd and muskmelon from Thailand having a bipartite genome (Ito et al., 2008).

Sponge gourd is being affected by Geminivirus, which was having the 89.6-95.1% sequence identity with tomato leaf curl New Delhi virus. The data suggest that the Begomovirus associated with the yellow mosaic disease of L. cylindrica is a putative strain of ToLCV-NDe (Sohrab et al., 2003).

Tiwari et al., (2012) studied the phylogenetic relationship among isolates under study and with other Begomovirus isolates reported from India. The isolates selected during the study were TLCNDV, ToLCPV, SLCCV and AgEnV Begomovirus species (at least one representative of each species). During the phylogenetic analysis, six Begomoviruses under the study and other selected Begomovirus isolates formed three main clusters and isolates of ToLCNDV and SLCCV species further formed two sub-clusters which clearly indicated the existence of four distinct clusters of Begomoviruses. They also found out based on highest sequence identities and closest phylogenetic relationships, that Begomoviruses were identified as isolates of AgEnV from pointed gourd (T. dioica); SLCCV from
pumpkin (C. maxima) and ToLCNDV from spongegourd (L. cylindrica), ridgegourd (L. accutangula) and from bittergourd (M. charantia). The virus isolates from pumpkin (C. pepo) was identified as ToLCPV.

**Diagnosis and detection**

**Infectivity Assay**

Indicator plants are used for detecting the presence of thrips that can transmit Tospoviruses. The pathogen quickly develops blackish lesions when infectious thrips feed on their leaves, thus revealing when and where infectious thrips occur in growing areas. Petunia cultivars Burgundy Madness and Blue Carpet were found to be good indicator plants for INSV and TSWV. Dark lesions developed on leaves of these petunias within about 2 to 4 days after they were fed by infectious thrips (Daughtrey *et al.*, 1997).

Yeh *et al.*, (1992) assayed the diseased samples of infected watermelon plants on test plants by mechanical inoculation (phosphate buffer containing sodium sulphite, (pH 7.0). The development of local lesions on C. quinoa and systemic symptoms of mottling, wilt and death of N. benthamiana was observed. When healthy watermelon seedlings were inoculated with TSWV- W, they showed symptoms of plant stunting, leaf narrowing, mottling, yellow spotting, and veinal and tip necrosis; these symptoms resemble those observed in field infected watermelon plants. When the virus from experimentally infected watermelons was transferred to C. quinoa and N. benthamiana, these hosts responded with local lesions and systemic infection, respectively, indicating that the virus recovered from field- infected watermelon is the causal agent of the disease. Reddy *et al.*, (1991) used cowpea and Petunia hybrida as diagnostic hosts, since they produced local lesions. On cowpea cv. C-152 and California Black Eye, GBNV produced concentric chlorotic lesions on the inoculated
leaves within 4 to 5 days after inoculation, while in petunia it produced necrotic lesions within 3 to 4 days after inoculation.

Krupashankar (1998) reported the success in WBNV transmission as 20 to 80% various plant species, showed to transmission of the virus mechanically using phosphate buffer (0.1 M, pH 7.0 and containing 0.02 M 2- mercaptoethanol). The virus produced chlorotic or necrotic lesions on cowpea, Chenopodium amaranticolor and Nicotiana rustica. Among these cowpea (cv. C-152) was a good assay host for watermelon bud necrosis virus since it produced local lesions within 4 to 5 days of inoculation.

Rao et al., (2003) used 0.05 M phosphate buffer (pH 7.0) containing 0.75 % thioglycerol for mechanical transmission of GBNV isolates from leaf curl symptoms of mungbean and urdbean to an indicator host cowpea (cv. C-152) and recorded chlorotic lesions. Inoculation on respective host symptoms resemble those observed on field infected plants. The results of the bio-assay of the necrosis virus affecting tomato revealed that the necrosis virus produced chlorotic local lesions on cotyledonary leaves of cowpea (V. anguiculata cv. C-152) within 4-5 days of post-inoculation Chlorotic and necrotic lesions were also recorded in Chenopodium amaranticolor (Anjaneyareddy et al., 2008).

Serodiagnostic techniques are widely used in the identification of virus and viral diseases. Although symptoms dictate the presence of the viral pathogen, but in absence of symptoms, the masked conditions does not prevail the presence of the viruses. In such cases, the viral pathogens are accurately detected by the various serological tests. The use of microtitre method of ELISA detection was demonstrated by Clark and Adams (1977).
On the basis of nucleocapsid protein (N) serology, GBNV, WBNV, and CaCV were grouped into WSMoV serogroup, IYSV into IYSV serogroup, and PYSV into unclassified serogroup and Tospoviruses are serologically indistinguishable (Fauquet et al., 2005). As purification of tospovirus is difficult, recombinant N protein expressed in *Escherichia coli* has been utilized for the production of PAb (Jain et al., 2005), which led to the commercialization of an ELISA-based diagnostic kit for GBNV and other serologically related tospoviruses in India (Mandal and Jain 2010). Monoclonal antibodies developed against N protein of GBNV were highly specific and capable of differentiating GBNV and WBNV isolates, and a simple dot blot assay was developed for the detection of GBNV in field samples (Hemalatha et al., 2008). An unequivocal identification of Tospovirus species is based on Ngene sequence with a threshold level of <90% amino acid sequence identity (Goldbach and Kuo, 1996). Reverse transcription–polymerase chain reaction (RTPCR) using virus-specific N-gene primers have been standardized and validated (Jain et al., 1998 and Raja and Jain 2006). Specific primers and RT-PCR methods were successfully used for the detection of GBNV and WBNV, which are otherwise serologically indistinguishable. Further, a single tube one-step RT-PCR method was developed using degenerate, conserved forward and virus-specific reverse primers for the specific detection of GBNV, WBNV, and CaCV (Kunkalikar et al., 2010). Daughtrey et al. (1997) suggested employing of different methods like infectivity assay, electron microscopic examination of infected samples, enzyme-linked immunosorbent assay (ELISA), direct tissue-blot assay, dot blot immunoassay and direct examination of plant tissues for characteristic viral inclusions for conformity of *Tospovirus* infection.
Immunoassay

An ELISA study was conducted for the identification of ZYMV, WMV-2 and PRSV-W by Davis and Mizuki (1987). Similarly, Yeh and Chen (1988) used Double Antibody Sandwich (DAS) ELISA to assay replication of PRSV at various intervals in *Cucumis metuliferus* in line ACC 2459 susceptible to PRSV and line 292190 highly resistant to PRSV and found that line 292190 were immune to the virus at all the growth stages.

Twenty-seven isolates of PRSV from Taiwan were found to be distinguishable with monoclonal antibodies in ELISA test, whereas, with polyclonal antibodies they were found indistinguishable (Kuan *et al.*, 1999). Rao (1999) reported that three Potyvirus groups gave moderate to strong reaction to all the potyvirus antisera used. Group-1 and 2 strongly cross reacted to PRSV-P and PRSV-W antisera, while group 3 showed strong reaction to WMV-1 antiserum in ELISA test. In ecological relationship study of PRSV with other viruses using DAC-ELISA conducted by Reddy (2000), PRSV reacted positively to all Potyvirus antisera but not with CMV antiserum, whereas, PRSV isolate reacted to both PRSV-P and PRSV-W antisera.

Serodiagnostic techniques are widely used in the identification of virus and viral diseases. Although symptoms dictate the presence of virus, but in absence of symptoms, the masked conditions does not prevail the presence of the viruses. In such cases, viruses are accurately detected by various serological tests. Plant viruses are immunogenic and were used as antigens for the production of antibodies and utilized in serological detection of viruses. The identification of the virus is best carried out by serological assays like immuno-diffusion and ELISA (Clark and Adams, 1977), rather than using biological properties such as host range and symptom expression on the indicator host, which may be confused with similar symptoms produced by other
viruses (Webster et al., 2004; Fisher and Nameth, 1997). The use of specific antiserum offered one of the most reliable criteria to identify plant viruses.

The use of microtitre method of ELISA detection was demonstrated by Clark and Adams (1977). Yemewar and Mali (1980) found that PRSV was not serologically related to bean common mosaic virus, bean yellow mosaic virus, cucumber mosaic virus, potato virus M, potato virus S and tobacco mosaic viruses, when tested with PRSV antisera. Yeh et al. (1984) found that isolates of PRSV and WMV-1 collected from different hosts were serologically indistinguishable in Agar Immuno Diffusion test with antisera of PRSV and WMV-1.4 An ELISA study was conducted for the identification of ZYMV, WMV-2 and PRSV-W by Davis and Mizuki (1987).

Six different leaf samples of Luffa acutangula with different symptoms were serologically detected by Dot Immuno Binding Assay (DIBA) of which, symptomatic samples such as fern leaf, mosaic, vein clearing and leaf curl were found positive against PRSV-P, where as, chlorotic spot and interveinal chlorosis were found positive against PRSV-W and WMV-2 (Kader et al., 1997). In Luffa operculata the natural incidence of PRSV-W was confirmed by Lima et al. (1997) using similar serological studies. Zouba et al. (1997) in their survey collected 716 samples of cucurbits infected with viruses and tested for their presence by ELISA. Results revealed the presence of WMV-2, ZYMV, PRSV-W, CMV, SqMV, Tomato Ring spot Nepo Virus, Tobacco Ring spot Nepo Virus and Tomato Spotted Wilt Tospo Virus. Similarly twenty-seven isolates of PRSV from Taiwan were found to be distinguishable with monoclonal antibodies in ELISA test whereas, with polyclonal antibodies they were found indistinguishable (Kuan et al., 1999). Rao (1999) reported that the three Potyvirus groups under study gave moderate to strong reaction to all the potyvirus antisera used, and groups 1 and 2 strongly cross reacted to PRSV-P and
PRSV-W antisera, while group 3 showed strong reaction to WMV-1 antiserum in ELISA test. In ecological relationship study of PRSV with other viruses using DAC-ELISA conducted by Reddy (2000), PRSV reacted positively with all Potyvirus antisera but not with CMV antiserum, whereas, PRSV isolate reacted to both PRSV-P and PRSV-W antisera.

Pandey et al. (2004) raised polyclonal antiserum against the WBNV N protein and which was able to detect watermelon bud necrosis as well as GBNV isolates collected from a wide range of hosts from different locations. Watermelon bud necrosis virus in India was also found to be similar to watermelon tospovirus from Japan and Taiwan and is closely related to Indian Peanut bud necrosis virus (PBNV), but is distinct from, or not related to the lettuce strain of TSWV and INSV (Singh and Krishnareddy, 1996). BND-infected samples reacted strongly to homologous antiserum by direct antigen coating (DAC) and protein A coated (PAC)-ELISA but failed to react with various TSWV and INSV isolates (Reddy et al., 1992) implying that the GBNV was found to be serologically distinct from TSWV and INSV. Based on DAC-ELISA the association of GBNV with crops like black gram, cowpea, green gram and soybean was confirmed (Bhat et al., 2001). Various other diseases such as spotted wilt in tomato (Rao et al., 1980) and leaf curl of mungbean and urdbean (Amin et al., 1985) were initially found to be caused by TSWV but, the causal agent was later confirmed as GBNV precisely using ELISA technique (Umamaheswaran et al., 2003; Rao et al., 2003).

In vitro gene expression strategy was used for the production of polyclonal antiserum to the nucleocapsid protein (N) of GBNV. The GBNV NP gene from cowpea isolates were cloned into 6x His-tagged UA cloning vector and expressed in *E. coli* cells. The fusion protein was detected in an insoluble fraction and was purified
by using Ni-NTA agarose resin. The purified 6x His-fusion protein (~32 kDa) were used for immunisation to produce a high titre polyclonal antiserum. The antiserum to the N for GBNV at 1:4000 dilutions detected successfully the natural infection of GBNV and WBNV in a wide range of cucurbitaceous, leguminous and solanaceous hosts from different locations (Jain et al., 2005).

**PCR based detection**

For rapid and sensitive detection of Begomovirus infection in cucurbits, nucleo-diagnostic methods have been developed. The nucleic acid hybridization test using radiolabelled probe to putative coat protein gene of tomato leaf curl New Delhi virus [Luffa] (ToLCNDV-[Luf]) detected Begomovirus in several cucurbits and whitefly. These methods were efficient in detecting Begomovirus in crude extracts of field samples. The polymerase chain reaction (PCR) was standardized using primer synthesized from Rep and coat protein [CP] genes of ToLCNDV-Luf. The CP gene based primers successfully detected Begomovirus in several cucurbits that were naturally infected.

**Taxonomy, classification and serological relationships**

**Tospovirus**: The genus Tospovirus within the family *Bunyaviridae* is comprised of 18 distinct species infecting a wide range of plant species (Moyer et al., 1999) and TSWV were classified as the type member of the *Tospovirus* genus (de Haan et al., 1990; Francki et al., 1991). Although the sequence homology between tospoviruses varied with the gene, sequence differences between the nucleocapsid (N) genes were accepted as a measure of overall relatedness. Isolates having N gene sequence homology to an extent of more than 90% were classified as strains of the same virus. Serologically related isolates with 80-90% sequence homology were classified as strains or distinct viruses depending on certain additional criteria. Isolates with less
than 80% homology to all of the other known viruses were classified as distinct viruses or species (Moyer et al., 1999). The representative positive samples of tospovirus isolates from different crops (tomato, chilli, groundnut, carrot, brinjal and watermelon) reacted strongly with GBNV and WSMoV antisera. None of the tospovirus isolates collected from tomato, chilli, groundnut, carrot, brinjal and watermelon showed positive reaction with TSWV, INSV, CSNV and IYSV antisera, with absorbance values below the threshold value (Bhanupriya, 2006).

**Begomoviruses:** In order to bring classification in the nomenclature of hundreds of Begomoviruses characterized, the study group on the taxonomy of geminiviruses suggested rules and guidelines for demonstrating species. These guidelines have been accepted by seventh International Committee on Taxonomy of Viruses (ICTV) which is as follows. A value of 89% identify in DNA a nucleotide sequence is kept as the threshold between the two species. Name of a virus species will give indication to the type of symptoms produced and the geographical location, from where it was isolated. For instance, Tomato leaf curl New Delhi, refers to a tomato Begomoviruses causing leaf curl symptoms isolated from New Delhi is characterized and sequence identity between ToLCNDV and new virus isolate is 89 or 91%, the new isolate is considered as the same species, and if the identity is less than 89% it is designated as a new species.