STUDIES ON BUD BLIGHT DISEASE OF TOMATO CAUSED BY GROUNDNUT BUD NECROSIS VIRUS

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1. INTRODUCTION

Tomato [*Solanum lycopersicum* (Mill.) Wettsd] is the second most important vegetable crop of Peru-Ecuadar origin (Rick, 1969) after potato and belongs to the family Solanaceae. In India, it was introduced by the English traders of East India Company in 1822. The name tomato probably derived from the word "Tomat" in the Nahua tonque of Mexico.

In India, it occupies an area of about 0.54 million hectares with production of 7.6 million tonnes with an average yield of 14.07 tonnes per hectare. In Karnataka, it occupies an area of 0.4 lakh hectares with a production of 1.14 million tonnes (Anon., 2006) with an average yield of 28.5 tonnes per hectare.

Tomato can be used as raw vegetable in sandwitches and salads; it can be processed to several products like puree, paste, soup, juices, ketchup, whole canned fruits *etc.* Tomato also has high medicinal value; the pulp and juice are digestable, promoter of gastric secretion and blood purifier. It is an important source of minerals and vitamin A and C. Tomatoes are important source of lycopene an antioxidant, ascorbic acid and β-carotene and valued for their colour and flavour. Tomatoes are called as "Poor man's apple".

The major tomato growing states in the country are Orissa, Bihar, Karnataka, Andhra Pradesh, Maharastra, Madya Pradesh, and Tamil Nadu. In Karnataka, major tomato growing districts are Kolar, Bangalore, Tumkur, Mysore and Mandya. Bangalore and Kolar districts produce nearly 35 per cent of total production in Karnataka (Anon., 2006). Though, the area under tomato cultivation is high, the productivity (20 tonnes/ha) is low, this attributed to the potential loss in yield due to number of diseases.

Besides fungal and bacterial infections, it is also affected by large number of viral diseases (Anon., 1983). Tomato is reported to be susceptible to over 40 viruses belonging to Alfamo, Luteo, Carla, Cucumo, Gemini, Poty, Illar, Nepo,Tombus, Tobamo and Tospovirus groups (Allen and Gibs, 1990). Among several viral diseases tomato leaf curl and tospoviruses are very important. The incidence of tospoviruses in vegetable crops is increasing year by year and more so in tomato (Krishna Reddy *et al.,* 1997).

In the world, tomato is known to be naturally infected by four different tospoviruses such as tomato spotted wilt virus (TSWV), tomato chlorotic spot virus (TCSC), groundnut ring spot virus (GRSV) and tomato Tospovirus (Heinze *et al.,* 1995). Tomato Tospovirus was reported from Taiwan, serologically closely related to watermelon silver mottle virus (WSMoV) and peanut bud necrosis virus (PBNV) (Adam *et al.,* 1993).

In India Tospovirus on tomato was observed for the first time from Niligiris in 1975 and reported as TSWV, later has been reported from Andhra Pradesh (Prasad Rao *et al.,* 1980), Karnataka (Sastry, 1982), Maharastra (Joi and Summanvar, 1986) and Tamil Nadu (Doraiswamy *et al.,* 1984). The occurrence of Tospovirus infection has been reported on several crops like chilli, brinjal, cowpea, potato, cluster bean, mungbean, urdbean, muskmelon, soybean, pea and peanut. In India host range and serological studies indicated that tomato Tospovirus is considered as a strain of GBNV and designated as a GBNV – To. The Tospovirus infection on tomato also called as bud blight, the species of groundnut bud necrosis virus (GBNV) identified as the causal agent of the tomato bud blight (Umamaheswaran *et al.,* 2003).

In Maharastra, Joi and Summanvar (1991) reported that the incidence of Tospovirus was found to be highest in *rabi* (22.3%) followed by *summer* (20.3%) and *kharif* (18.6%). Singh and Tripati (1991) reported the incidence of Tospovirus on tomato upto 30 per cent in Northern India. Plants infected with Tospovirus caused yield loss of 100 per cent if infection occurred within 30 days of planting.

Tospovirus is the only genus of the family Bunyaviridae that infects plants (Murphy *et al.,* 1995; Fauquet *et al.,* 2004). Tospovirus virions are quasi – spherical, enveloped and contain three linear ssRNA species, denoted as small (S) RNA, medium (M) RNA and large (L) RNA (Mohammed, 1981; Van Den Hurk *et al.,* 1977; Van poelwijk *et al.,* 1993).

In India, groundnut bud necrosis virus (GBNV), peanut yellow spot virus (PYSV) and watermelon bud necrosis virus (WBNV) have been reported to infect a broad range of vegetables (Tomato, potato, chilli, peppers, and watermelon). The tomato Tospovirus is now increasing in India on tomatoes, it occurs at high proportions in some states especially in the states of Tamil Nadu, Karnataka, Maharashtra and Andhra Pradesh. The losses due to this disease in tomatoes depends mainly on the level of incidence stage of infection and severity of symptoms. Flowering and fruit formation stage is susceptible to this virus.

In Karnataka, the work with regard to Tospovirus on tomato is scanty. Therefore, there was a need for detailed study of this disease in respect of transmission, host range, serological relationship with other Tospoviruses, characterization of the causal agent and management of the disease. Hence, the present studies have been undertaken with the following objectives.

1. Survey in different tomato growing areas for the disease incidence of Tospovirus
2. Diagnosis of disease through

   (a) ELISA, (b) PCR, (c) Cloning and sequencing (d) Electron microscopy
3. Transmission studies

   a. Mechanical transmission b. Vector transmission
4. Host range studies
5. Management of bud blight disease

a. Screening of tomato varieties b. Antiviral resistance

c. Disease management in the field

2. REVIEW OF LITERATURE

2.1 HISTORICAL ACCOUNT

The "tomato spotted wilt disease" was first observed in tomato fields of Australia during 1916 (Brittlebank, 1919) and it was shown to have viral etiology (Samuel *et al*., 1930). The disease was reported to be transmitted by thrips (Pittman, 1927) and the causal agent described as "Tomato spotted wilt virus" (TSWV). However, several reports have led to some confusion due to reporting the same virus under different names, ex: tomato bronzing virus, kromnek virus, pineapple yellow wilt virus, makhorka tip chlorosis virus, vira cabeca virus and others (Best, 1968; Smith, 1972; Sakimura, 1962). Variation in nomenclature reflects wide variation in disease symptoms, host species and the geographical regions where the disease has been reported. TSWV is known to infect 1090 different plant species, belonging to more than 85 distinct botanical families (Sherwood *et al.,* 2000; Parrella *et al*., 2003).

The occurrence of Tospovirus infection has been reported in India on various crops like peanut (Ghanekar *et al.,* 1979), tomato (Prasad Rao *et al.,* 1985), peas (Prasad Rao *et al.,* 1984), urd bean and mung bean (Amin *et al.,* 1985), cowpea, chilli, egg plant and cluster bean (Krishna Reddy and Varma, 1990).

The disease was first observed in tomato variety marglobe in Nilgiri hills during 1964 and the ripening fruits exhibits spots with circular markings as concentric bands of red and yellow broken rings about one cm in diameter (Todd *et al.,* 1975). The disease is characterized by bronze or purple coloured leaves, severe necrosis of buds, petioles, and pale yellow or red concentric rings turning into necrosis on fruits (Raja and Jain, 2006).

The disease has recently become important in hybrid tomatoes in Karnataka, Kerala, Maharastra, Tamil Nadu, and Uttar Pradesh and incidence ranging from 19-34 per cent has been recorded. The disease incidence is more in Kharif and summer seasons as compared to rabi season (Anon, 2004).

Sastry (1982) reported the occurrence of tomato Tospovirus on tomato in Karnataka. The symptoms observed on tomatoes in the field were tip necrosis, necrotic spots on leaves, petiole and stems of the infected plants, finally wilting of the plants. Infected plants produced fruits often showing chlorotic concentric rings.

2.2 DISEASE INCIDENCE

Incidence of tomato Tospovirus in recent years has assumed a serious proportion in Maharashtra occurring throughout the year in a severe form and causes heavy losses in yield (Joi and Summanavar, 1986). During 1980s, TSWV caused significant losses on major crops like groundnut, tobacco and tomato in the South Eastern United States, Eastern Europe and South America. TSWV is the most destructive plant virus and it was estimated that world wide it causes losses, mainly in commercial vegetable crops of around one billion US $ annually (Scott, 2000).

Joi and Summanvar (1991) studied the effect of seasonal variation on thrips population and its subsequent effect on variation of TSWV incidence on tomato and groundnut. The incidence of groundnut and tomato was highest in *rabi* (20.8 and 22.3 per cent respectively), summer (17.6 and 20.3 per cent respectively) and *kharif* (4.8 and 18.6 per cent respectively). The thrips population density was 26 per groundnut plant and 14 per tomato plant.

Kumar and Irulappan (1991) studied the TSWV infection in tomato, at different growth stages in different varieties, the plants infected at 30 days of planting showed 100 per cent loss when compared to 45 and 60 days of planting. This shows earlier stages of infection the greater reduction in yield in all the varieties. Samuel *et al.* (1930) also observed total loss in tomato if TSWV infection occurred within ten weeks after transplanting.

Fajardo *et al.* (1998) observed that the disease gradient ranging from 23.4 per cent at 12.5 m from pea crop (Centre row of the first tomato plot) down to 5.9 per cent at 637.5 m

(Centre row of the lost tomato plot). The decrease in incidence from the source fit to on exponential regression strongly indicated a high dispersal incidence of tospoviruses from pea crop, which was considered the source of tospoviruses and viruliferous thrips. A marked yield reduction was observed in plants showing mild disease symptoms (57.1%) and in plants showing severe symptoms (84.4%) with regard to total yield per plant.

The yield loss due to GBNV in India was estimated to be more than 80 per cent (Das Gupta *et al.,* 2003). The annual loss due to GBNV in Asian countries was estimated to be exceeding US$ 89 million (Pappu, 1997).

The virus seriously affected soybean cultivation, particularly in Madya Pradesh (Thakur *et al.,* 1998). While the mung bean leaf curl disease caused by GBNV in the Telangana region was upto 19 per cent and 34 per cent during *kharif* seasons of year 2000 and 2001, respectively and the disease on urd bean was upto 12 per cent in the *rabi* season of 2001-02 (Prasad Rao *et al.,* 2003).

2.3 VIRUS DETECTION

2.3.1 Biological detection

Cowpea cultivar C-152 was used as a virus indicator assay host where the inoculated leaves produced chlorotic lesions (Ghanekar *et al.,* 1979, Prasada Rao *et al.,* 1985, 1987, 2003; Bhat *et al.,* 2002). However, Bhat *et al.* (2002) has used a different cowpea cultivar, Pusa Komal. Reddy *et al.* (1990) used cowpea and *Petunia hybrida* as diagnostic hosts as both produced local lesions. On cowpea cv C-152 and California Black Eye, GBNV produced concentric local lesions on the inoculated leaves within 4 to 5 days after inoculation, while petunia produced necrotic lesions in 3 to 4 days after inoculation.

2.3.2 Serological detection

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 by serological assays like immuno diffusion and enzyme linked immuno sorbent assay (ELISA) (Clark and Adam, 1977), rather than using biological properties such as host range and indicator host, which may be confused with similar symptoms produced by other viruses (Webster *et al.,* 2004; Fisher and Nameth, 1997). Based on DAC-ELISA, the association of GBNV in black gram, cowpea, green gram and soybean was confirmed (Bhat *et al.,* 2001). Various other diseases initially characterized to be caused by TSWV such as spotted wilt in tomato (Prasad Rao *et al.,* 1980) and leaf curl of mung bean and urd bean (Amin *et al.,* 1985) were confirmed as GBNV precisely using ELISA technique (Umamaheshwaran *et al.,* 2003; Prasad Rao *et al.,* 2003).

Serological relatedness of tospoviruses implies sharing of epitopes on the single capsid protein. Serological relationship between viruses may indicate that one or more of the three major structural proteins (N,G1, G2) were serologically related with little or no relationship between the other structural proteins or a sharing of epitopes at individual proteins. The relations were generally established using polyclonal antisera made against infectious virions (German *et al*., 1992).

A Tospovirus isolate Tospo-To, was recently isolated from tomato in Taiwan, it was shown to be the serologically related to WSMoV (Taiwan), WSMoV (Japan) and PBNV (India) (Adam *et al.,* 1993). All three isolates have similar N protein larger than other tospoviruses. The sequence comparisons of N protein amino acid sequence of several different Tospovirus species indicated that Tospo-To has 99 per cent similarity with WSMoV, 32 per cent With TSWV and 26 per cent TCSV, 33 per cent with GRSV and 30 per cent INSV (Heinze *et al.,* 1995).

Comparison of WSMoV and PBNV have indicated that these two viruses were closely related, they share a high level of serological cross reaction and both possess on N protein of 32 KD, compared to 29 KD for TSWV and INSV (Adam *et al.,* 1993). The N protein of PBNV has 86 per cent identity with that of WSMoV and approximately 30 per cent with TSWV more

over, WSMoV induced severe systemic symptom in most of *cucurbit* sp. and does not infect peanut while PBNV causes systemic infection in peanut but local infection in cucurbits (Reddy *et al.,* 1992, Yeh and Chang, 1995). Although, more serological relationship between the two N proteins suggests that, WSMoV and PBNV are two strains of other same species in sero group IV, (Adam *et al.,* 1993, Reddy *et al.,* 1992).

Raja and Jain (2006) reported that the bud blight affected samples collected from Coimbatore, Pune, Rahuri showed positive reaction with polyclonal antiserum to nucleocapsid protein (N) of Groundnut bud necrosis virus (GBNV) in direct antigen coated enzyme linked immuno sorbent (DAC-ELISA)and dot immuno binding (DIBA) assays, suggesting that the association of a Tospovirus isolated antigenically related to GBNV.

The leaf and petiole samples from typical symptomatic tomato plants reacted with poly antiserum (PBNV) using DAC-ELISA detected in all the different plant parts and absorbance values (A 405 nm) varied from 0.01 (rind of ripened fruit to 1.292 flower by Reddy *et al.* (2008).

2.3.3 PCR based detection

PCR based techniques are extremely sensitive and provide additional advantage that the amplican can be sequenced to know more about the molecular details (Webster *et al.,* 2004). Several PCR based methods have been developed for specific detection of TSWV; first PCR based assay was developed by Mumford *et al.,* (1994). Immunocapture (IC) PCR and RT-PCR developed by Nolasco *et al.,* (1993) and Weekes *et al.* (1996), respectively for detection of tospoviruses. Several universal primer sets have been designed to do PCR tests for identification and characterization of distinct tospoviruses in infected plants and thrips.

Degenerate primers designed from conserved regions of L genes from TSWV, impatiens necrotic spot virus (INSV), water melons silver mottle virus (WSMoV) and GBNV isolate were used to amplify the corresponding regions of L genes from seven distinct tospoviruses (Chu *et al.,* 2001).

Jain *et al.* (1998) designed degenerate primers based on the conserved nucleotide homology of the NP gene sequence from GBNV and WSMoV and used for molecular diagnosis of GBNV and WBNV isolates in India.

Ronco *et al.* (1989) used complementary DNA (cDNA) probe prepared for viral RNA for TSWV detection. Jain *et al.,* (1998) conducted reverse transcription PCR (RT-PCR) to achieve rapid and reliable amplication of TSWV sequence from peanut both leaf and root tissue of peanut isolates with less than 80 per cent homology to all of the known viruses are classified as distinct viruses. Those viruses exhibiting serological relatedness are classified in the sero group (Law and Moyer, 1990).

De Avila *et al.* (1990) reported that INSV, TCSV and GRSV were serologically distinct from TSWV, the level of variation is very less with a greater degree of cross reactivity between viruses. This greater level of relatedness can be attributed to a higher degree of similarity at the amino acid level, with 76 per cent and 78 per cent homology between the N proteins of TCSV and TSWV and GRSV and TSWV respectively. Between the N proteins of TCSV and GRSV, the amino acid homology in 81 per cent (De Avila *et al.* 1993a. 1993b).

Peanut bud necrosis virus (PBNV), initially reported to be caused by TSWV (Ghanekar *et al.,* 1979), the causal agent now being identified as a serologically distinct Tospovirus (Reddy *et al.,* 1992) and has been renamed as PBNV (Adam *et al.,* 1993) the causal agent of watermelon Silver mottle virus reported as TSWV-W from Japan (Iwaki *et al.,* 1984) and Taiwan (Yeh *et al.,* 1992) now it is considered to be a distinct Tospovirus named as watermelon silver mottle virus (WSMoV) (Yeh and Chang 1995) it was significantly different from TSWV and INSV which do not systemically infect members of the cucurbitaceae (Yeh *et al.,* 1992). The nucleotide sequence of N gene of WSMoV has been determined revealing a nucleic acid sequence homology of around only 55 percent with all the Tospovirus members compared and even less (42-46%) at the amino acid level (Yeh and Chang, 1995).

Rang Lal Meena *et al.* (2005) reported the presence of Tospovirus in thrips vector, *Scirtothrips dorsalis* has been detected for using RT-PCR analysis. Similarly RT-PCR analysis with GBNV infected tomato leaves also resulted in the amplification of DNA corresponding to the N gene (~ 830 bp) of Coimbatore isolate. Dendrogram constructed on the basis of RAPD similarity matrix revealed that *S. dorsalis* population from tomato, groundnut and chillies had at least 75 per cent similarity while, only 50 per cent similarity existed between the *Frankliniella schultzei* population from cowpea and sunhemp. *Thrips tabaci* from cotton was distantly related with *S. dorsalis* and *F. schultzei* with lowest similarity indices
(< 0.464).

Reddy *et al.* (2008) reported that association of a Tospovirus in causing necrosis in tomato was confirmed by amplifying N-gene of GBNV with specific primers. The RT-PCR successfully amplified the N-gene from tomato samples collected from symptomatic host plants and this suggested the association of an isolate of GBNV with tomato necrosis disease.

2.3.4 Cloning and sequencing

Umamaheswaran *et al.* (2003) reported that Tospovirus was isolated from tomato plants showing severe necrosis of leaves and growing buds. The host range studies and sequence analysis of nucleocapsid protein (N) gene showed that the amino-acid sequence identities with other tospoviruses were in the range of 16-85 per cent and it is concluded that the tomato Tospovirus should be considered as a strain of GBNV and designated as GBNV-To.

Akram *et al.* (2004) 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 sequence analysis revealed that the NSM gene was highly conserved among Indian GBNV isolates.

2.3.5 Electron Microscopy (EM)

Detection of virus particles and their identification by EM can usually be achieved by examination of leaf dip preparations or thin sections of infected cells (Milne, 1970). Fixation is found to be essential to avoid flattening and distortion of particles before staining (Francki and Halta, 1981). Ammonium molybdate is found to be a good negative stain. The isolated particles lack uniformity of size and shape and hence are not readily distinguished from some normal cell materials. The problems associated with negative staining of TSWV are due to the presence of viral envelope. Preparation of thin sections from infected tissues of the plant, though time consuming and requires skill, still is recommended as a very reliable method of identifying TSWV. The particles in section are relatively uniform and cannot be mistaken for those of any other virus. In addition, presence of viroplasm in many of the cells is characteristic of TSWV infection (Francki and Halta, 1981).

Reddy *et al.* (1990) examined thin sections of infected leaf tissue with electron microscope, which revealed that virus particles were associated with endoplasmic reticulum. Several particles were seen clustered in the cisternae of endoplasmic reticulum. They reported that crude plant extracts and purified preparation should be fixed in 1.5 per cent glutaraldetyde and negative staining with 1 per cent uranyl acetate was preferable to the use of phospho tungstate or ammonium molybdate.

Purified preparation on carbon coated copper grids, negatively stained with 2 per cent uranyl acetate, when examined in Jeol 100B electron microscope at 80 KV revealed spherical particles of 75 to 85nm diameter. There were a few or no membranous contaminants present and the virus particles had a well-defined, unstained envelope surrounded by an outer layer of projections. No internal structure could be differentiated even in particles penetrated by stain (Mohammed, 1981).

2.4 TAXONOMY

Tospoviruses in general possess a quasi-spherical, enveloped virion containing a tripartite RNA genome. An open reading frame (ORF) in the viral complementary sense (negative) is located on the large RNA (L) segment; two other ORFs in ambisense orientation are on the middle (M) and small (S) RNA segments. The presence of two ambisense RNA segments distinguishes this genus from other genera in the *Bunyaviridae* (Moyer, 1999).

2.5 HOST RANGE

TSWV has an extremely wide host range exceeding 500 species of plants covering 70 families of both monocotyledons and dicotyledons (Gold Bach and Peters, 1994).

Under experimental conditions, Susceptibility to infection by Tospovirus was found in many plant families. Susceptible host species are found in the family Amaranthaceae, Amaryllidaceae, Apocynace. Balsaminaceae, Bromeliaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Iridaceae, Leguminosae-Caesalpinioideae, Leguminosae-Papilionoideae, Malvaceae, Papavaraceae, Polemoniaceae, Solanaceae and Tropaeolaceae (Parrella *et al.,* 2003).

Ghanekar *et al.* (1979) reported that the bud necrosis disease of groundnut could induce chlorotic and or necrotic local lesions in some hosts and on others, it could produce chlorotic or necrotic spots followed by systemic infection. The same hosts were produced similar type of symptoms for TSWV on tomato (Prasad Rao *et al.,* 1980)

Cho *et al.* (1986) surveyed the major vegetable growing areas of Hawaii, sampled 9000 plant species, and found that 44 species were infected with TSWV. Smith (1987) reported the number of crop plant hosts of TSWV, which includes tomato, tobacco, pepper, begonia, dahlia, pineapple, potato, mung bean, lettuce, papaya, gladiolus, petunia and pea. Cho *et al.,* (1987) listed 237 host plants susceptible to TSWV from different parts of the world.

Sutha *et al.* (1998) reported that among the local lesions hosts, *Vigna unguiculata* produced 40 local lesions per leaf followed by *Chenopodium amaranticolor* with 32 local lesions. Among the systemic hosts, *Nicotiana clevelandi* recorded 90 per cent infection followed by *N. glutinosa* (80%), *Datura stramonium* (78%). Similarly, the extracts from these plants when inoculated to tomato, 78 per cent transmission was obtained from *V. unguiculata* and 52 per cent from *C. amaranticolor* and concluded that *V. unguiculata* could be used to maintain TSWV inoculum in the glass house as the most suitable host for its multiplication.

Krishnaveni *et al*. (2004) reported the host range for six different GBNV isolates collected from different crop species viz., tomato, groundnut, blackgram, pea, chilli, and brinjal out of 31 plant species tested by mechanical inoculations. Only 10 plant species *viz*., lima bean, green gram, soybean, black gram, groundnut, tomato, brinjal, chilli, and tobacco expressed visible symptoms for all the six isolates. However, irrespective of the isolate inoculated, 20 plant species showed positive reaction to GBNV by sap inoculation. None of the isolates expressed symptoms or showed positive reaction upon inoculation of beetroot, sunflower, safflower, cabbage, snake gaurd, bottle guard, okra and potato. On sap inoculation, the virus produces concentric chlorotic and necrotic local lesions on inoculated primary leaves of cowpea (*Vigna unguiculata* (L) walp.) variety C-152 and Var. Early Ramshorn.

GBNV induced chlorotic and or necrotic local lesions in *Beta vulgaris, Cajanus cajan* (Accession No, ICRISAT 2376), *Chenopodium amaranticolor, C. quinoa, Crotolaria juncea, Cucumis sativus,* (cv. national pickling). *Gomphrena globosa, Nicotiana rustica, N. tabaccum.* "Xanthi-nc," *Petunia hybrida* (cv. Coral Satin), and chlorotic or necrotic spots followed by systemic infection in *Canaralia ensiformis*, *Datura stramonium, Dolichos uniflorus, Glycine max* (cv. Bragg), *L. esculentum* (cv. Pusa ruby, and perfection). *Nicotiana clevlandii, N. glutinosa* a hybrid between *N. glutinosa* and *N. clevelandii, Phaseolus vulgaris* (cv. Boundiful and Top crop), *P. lunatus* (cv. Heriderson Bus Lima), *Physalis floridana, Pisum sativum* (cv. Bonneville), *Vigna unguiculata* (cv. C-152 and California black

eye), *V. radiata* (cv. Hy. 45), *V. mungo* (cv. UPU) *Vinca rosea* and *Zinnia elegans* (Ghanekar *et al.,* 1979).

2.6 TRANSMISSION

2.6.1 Mechanical transmission

Symptoms of tomato Tospovirus occurring in India include necrosis of the buds, chlorotic and streaks on petioles and stem. The ripening fruits exhibit spots with circular markings about 1 cm in diameter as concentric bands of red and yellow broken rings (Brittle bank 1919 and Todd *et al.,* 1975).

Prasad Rao *et al.* (1980) Succeeded in transmitting the virus by sap inoculation. Mechanical inoculation were made by grinding young infected leaves with cold 0.05M phosphate buffer (pH 7.0) containing 0.02 M, β-mercaptoethanol.

Ghanekar *et al*. (1979) was able to transmit efficiently the virus causing bud inoculation of the young infected cowpea cv. C-152 leaves using 0.05M phosphate buffer, pH 7.0 containing 0.02M β-mercaptoethanol and indicated that the other antioxidants like 0.01m cysteine hydrochloride and the chelating agent 0.01M sodium diethyldithiocarbamate were effective but inferior to 0.02M β-mercaptoethanol as assessed by the number of local lesions produced on cowpea.

Reddy *et al.* (1992) used 0.05 M potassium phosphate, pH 7.0 containing either 0.02M β-mercaptoethanol or 0.75 per cent (v/v) mono-thioglycerol in mechanical transmission of GBNV to cowpea and groundnut.

Prasad Rao *et al.* (2003) used 0.05M phosphate buffer, pH7.0 containing 0.75 per cent thioglycerol for the transmission of GBNV isolates from leaf curl diseased mung bean and urd bean to cowpea and the respective host species.

Bhat *et al*. (2002) mechanically transmitted the soybean isolate to cowpea using 0.1M phosphate buffer, pH 7.2 containing 0.1 per cent (v/v) β-mercaptoethanol. Predisposition of plants by keeping them in darkness for 24 hours prior to inoculation aids better transmission (Halliwell and Philley, 1974).

2.6.2 Thrips transmission

Tospoviruses are exclusively vectored by thrips. Pittman (1927) was first to establish that onion thrips (*Thrips tabaci*) transmitted TSWV on tomato. Seven other species of thrips namely; *Frankliniella fusca* (Hinis), *F. occidentalis* (Pergande), *F. schultzei* (Try born), *Scirtothrips dorsalis* (Hood), *Thrips palmi* (Karny), *T. setosus* (moulton) and *F. intonsa* (Tryborn) are reported as vectors of tospoviruses (Kormelink *et al.,* 1994). One of the most unique features of the thrips – Tospovirus relationship that only adults that acquired the virus at the first larval stage are able to transmit (Sakimura, 1963). While both larval and adult thrips transmit the virus in a persistent manner. Widely varying acquisition and inoculation thresholds latent periods, with durations of only five minutes have been reported (Sakimura, 1963) which enhance the chance of becoming more number of infected plants tospoviruses are circulative and also propagative. Though the thrips virus interactions underlying this variation is not clearly known (Best, 1968), but recent evidences suggests that the virus replicated in the midgut epithelia, muscle cells adjacent to the gut and in salivary glands (Ullman *et al.,* 1995).

Of all the Tospovirus vectors, the western flower thrips *Frankliniella occidentalis* (WFT) is considered as the most important. WFT is a highly efficient vector of tospoviruses capable of transmitting. TSWV, INSV, GRSV and TCSV (Gold bach and peters, 1994) in the western states of USA. In India, PBNV isolates were found to be transmitted by *F. schultzei* and *Scirtothrips dorsalis* have been reported to transmit TSWV in India (Todd *et al.,* 1975 and Prasad Rao *et al.,* 1980). More recently, *Thrips palmi* has also been shown to act as a vector of PBNV and WSMoV isolates (Yeh *et al.,* 1992).

Transmission is the result of the different processes that start with the ingestion of the virus on infected plants and end with the successful transmission of virus to a healthy plant. These events coincide with the host finding and feeding activities of the thrips. The ratio at which thrips become viruliferous and transmit can be quantified in terms of the efficiency with which the virus can be acquired and subsequently transmitted and the less of latent period. Its dynamics are the results of complex interactions between plants, vectors and viruses (Peters *et al.,* 1996).

2.7. MANAGEMENT OF THE DISEASE

2.7.1 Antiviral Principles

The application of antiviral principles extracted from the leaves of sorghum and coconut was found to be equally effective in reducing the disease incidence when compared to insecticides (Narayanaswamy *et al.,* 1983). Studies on the characterization of AVP from sorghum leaves indicated it to be a proteinaceous compound and the translocation mechanism might afford protection against virus infection. Sorghum AVP was found to inhibit lesion development effectively for a period of 10 days after application (Narayanswamy and Ramaiah, 1983).

The mode of action of antiviral principles has been studied in detail and it is reported that AVPs may inhibit polypeptide synthesis (Owens *et al.,* 1973), may produce virus inhibitory substances in the host which compete for virus infection sites (Van Kammen *et al.,* 1961) and may induce resistance in susceptible cultivars (Narayanaswamy and Ganapathy, 1986; Mukherjee *et al.,* 1982; Rao *et al.,* 1985; Narayanswamy and Ramaiah, 1983).

The AVP were found to be more effective as pre inoculation treatments than as post inoculation treatments (Aiyanathan and Narayanaswamy, 1988; Saigopal *et al.,* 1986; Straub *et al.,* 1986).

2.7.2 Screening for Tospovirus resistance

In tomato, the first resistance was found in *Lycopersicon pimpinellifolium* (Samuel *et al.,* 1930) and *L. esculentum* (Holmes, 1948) determined that those resistance were controlled by five genes, two dominant and three recessive. This resistance proved germplasm screening has been made in different *lycopersicon* species in order to find higher and non-isolate specific resistance. Some field resistance was detected in accessions of *L. hirsutum* and in *L. hirsutum* var glabratum. In field trials, it was found that some of these resistances were controlled by polygenic systems.

Mandal *et al*.(2006) Reported that the tomato cultivar BHN 444 suppressed the systemic symptoms caused by the severe isolate GAL and both local and systemic symptoms of the mild isolate GAT b-1 as determined by visual observation and ELISA and they indicated that BHN 444 has a high level of resistance against both GATb-1 and GAL isolates.

Jaya Jasmine and Seemanthini (1994) observed that none of varieties screened was fully resistant; however, they showed varied response to the infection. ARTH-4 among the hybrids and CO.3 among the varieties showed less susceptibility while the rest were highly susceptible. Similar results were obtained by Joi and Summanvar (1989).

L. peruvionum appears to be the best resistance source for TSWV, NPH accessions 201 and 374, LA ac cessions 111372, 385, 4441-1 and 1113-1, PI accessions 126928, 126930, 126, 944, 126946, 128657, 128600 and 129146 have been resistant or immune in screening trials (Paterson *et al.,* 1989; Maluf *et al.,* 1991; Iizuka *et al.,* 1993; Krishna Kumar *et al.,* 1993). The resistance derived from PI – 128657 introgressed in Anahu cultivar disappeared partially during the breeding process (Paterson *et al.,* 1989 and Krishna Kumar *et al.,* 1993) Contrarily, the resistance conferred by Sw-5 gene (Stevens *et al.,* 1992) introgressed in Stevens cultivar has been the preferably used by breeders. The resistance is isolate unspecific, easy to manage (dominant monogenic control) and shows good expression in *L.esculentum* background. Under high inoculation pressure in thrips transmission, partial over coming of this resistance has been found (Rosello *et al.,* 1997).

Reddy *et al.* (2008) reported that out of 33 arieties/hybrids none of them were immune to Tospovirus but only 2 were found to be resistant (Alkabasa V and PKM 1).

2.7.1.1 Induced systemic resistance

Induced resistance is defined as an enhancement of the plants defensive capacity against a broad spectrum of pathogens and pest that is acquired after appropriate stimulation. The resulting elevated resistance due to an inducing agent upon infection by a pathogen is called induced systemic resistance (ISR) or systemic acquired resistance (SAR) (Hammerschmidt and Kuc, 1995). The induction of systemic resistance by Rhizobacteria is referred as ISR, where as that by other agencies is called SAR (Van Loon *et al.,* 1998). Once resistance is induced, it will non-specific protection against pathogenic fungi, bacteria and viruses.

A large number of defense enzymes have been associated with ISR *i.e.* phenylalanine ammonia lyase, peroxidase, polyphenol oxidase and proteinase inhibitors (Van Loon, 1997). These enzymes also bring about liberation of molecules that elicit the first steps of induction of resistance, phytoalexins and phenolic compounds.

2.7.1.2 Phenylalanine ammonia lyase (PAL)

PAL catalyzes the deamination of L-phenylalanine to trans –cinnamic acid which is the first step in the bio sysnthesis of large class of plant natural products based on the phenylpropane skeleton, including lignin monomers as well as certain classes of phytoalexins. PAL activity also generates precursors of lignin biosynthesis and other phenolic compounds that accumulate in response to pathogen infection (Klessing and Malamy, 1994). PAL is the key enzyme in inducing synthesis of Salicylic acid (SA) which induces systemic resistance in many plants.

2.7.1.3. Peroxidase (PO)

Peroxidase have been implicated in the regulation of plant cell elongation, phenol oxidation, polysaccharide cross-linking, IAA oxidation, cross–linking of extension monomers and oxidation of hydroxyl – cinnamyl alcohols into free radical intermediates and wound healing (Vidhyasekaran *et al.,* 1997). These enzymes are also part of the response of plant defense to pathogens (Hammerschmidt and Kuc, 1995).

2.7.1.4 Polyphenol oxidase (PPO)

PPO usually accumulated upon wounding in plants. Biochemical approaches to under stand PPO function and regulation are difficult, because the quinonoid reaction products of PPO covalently modify and cross-link the enzyme PPO can be induced via, octadecanoid defense signal pathway (Constabl *et al.,* 1995).

In tomato, PPO is induced by caterpillar feeding, jasmonates and mechanical damage but not by mites or leaf miners (Thaler *et al.,* 1996). Similarly increased activity of PPO was observed in tomato by fluorescent pseudomonads in response to infection by tomato spotted wilt virus (Kandan *et al.,* 2002).

2.7.3 Management in field

Reddy *et al.* (1995) have reported that cultural practices such as adjustment in planting dates, maintenance of inter crops such as maize and pearlmillet could reduce incidence of peanut bud necrosis disease. These practices have shown to reduce infestation by *Thrips palmi*.

Momol *et al.* (2002) reported the metalized mulch was most effective in reducing disease incidence. Actigard a systemic acquired resistance induced reduces the incidence of tomato spotted wilt virus on the standard black mulch but not on metalized mulch spinosad was as effective as methamidophos in reducing the spread of the disease of metalized mulch, Actigard, and insecticides reduced tomato spotted wilt virus by as much as 76 per cent.

3. MATERIAL AND METHODS

The present investigations on bud blight disease of tomato caused by groundnut bud necrosis virus (GBNV) were carried out during 2006-08. The materials and methods followed during the course of investigation are presented in this chapter.

3.1 SURVEY IN DIFFERENT TOMATO GROWING AREAS FOR THE DISEASE INCIDENCE OF TOSPOVIRUS

The roving survey was conducted to know per cent disease incidence of bud blight disease of tomato and prevalence of thrips populations in four major tomato growing areas of Karnataka *i.e.* Dharwad, Haveri, Belgaum and Kolar districts during summer 2008. In each village five fields were selected and the percentage of disease incidence was assessed by recording the number of plants showing disease symptoms and the total number of plants examined by using the formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

While surveying data on variety grown, age of the crop other insects present, plant protection measures taken, percent disease incidence and also type of symptoms produced were recorded.

3.2. DIAGNOSIS OF DISEASE BY ELISA / PCR / CLONING / EM AND SEQUENCING

3.2.1 Enzyme Linked Immuno Sorbent Assay (ELISA)

For serological studies polyclonal antibodies PBNV obtained from ICRISAT, Hyderabad was used for DAC-ELISA.

Direct antigen coating (DAC) indirect ELISA was performed as per the procedure of Hobbs *et al.* (1987) and included the following steps. The various buffers used in ELISA were prepared as follows.

A) Coating buffer (Carbonate buffer) (pH 9.6):

Sodium carbonate (Na_2CO_3)	1.59 g
Sodium hydrogen Carbonate ($NaHCO_3$)	2.93 g
Sodium azide	0.2 g
Distilled water	1.0 L

The diethyl dithiocarbamate (sodium salt) was added at 0.001 M concentration (1.71 g/L).

B) Phosphate buffer saline (PBS) pH 7.4:

Disodium hydrogen phosphate (Na_2HPO_4, 2 H_2O)	2.9 g
Potassium di hydrogen phosphate (KH_2PO_4)	0.2 g
Potassium chloride (KCl)	0.2 g
Sodium chloride (NaCl)	8.0 g
Distilled water	1.0 L

C) Washing buffer (PBST):

PBS	1000 ml
Tween-20	0.5 ml

D) Antibody buffer:

PBS-Tween	100 ml
Polyvenyl Pyrrolidone (40000 MW)	2.0 g
Bovine Serum Albumin (BSA)	0.2 g

E) Substrate buffer (pH 9.8):

Diethanalamine	97 ml
Distilled water	800 ml
Sodium azide	0.2 g

pH was adjusted to 9.8 and the volume was made upto one litre. Substrate used was p-nitrophenyl phosphate (PNPP) when dissolved in substrate buffer it gave colourless solution. PNPP was dissolved in diethanolamine buffer just prior to use at 1.0 mg/ml and the substrate mixture was stored in dark by covering the substrate container with aluminium foil.

Procedure for DAC- ELISA

1. Crude extract was prepared in coating buffer-using dilution of 1:10 (1 gm of leaf sample/9ml of buffer). Extract was dispensed into each well of microlitre plate at the rate of 200µl per well using a micro pipette and the plates were incubated at 37°C for 2-2.5 hr.

2. The contents of the plates were poured off and rinsed in PBS-Tween. This was followed by washing the plates in three changes of PBS-Tween buffer allowing 3 min. for each wash.

3. Addition of antibody diluted in conjugate buffer to a concentration of 1:1000 dilution at the rate of 200 µl per well. Then the plates were incubated at 37°C for 2 to 2.5 hr and were washed in PBS – Tween as in step -2.

4. Alkaline phosphatase labelled antirabbit IgG diluted in antibody buffer to a concentration of 1:8000 dilution. The plates were incubated at 37^0C for 2hrs and were washed in PBS-Tween as in step-2.

5. Substrate buffer containing 0.5-1mg/ml of PNPP was then dispensed to each well @ 200 µl/well and incubated at room temperature for 10-30 minutes. Light orange to yellow color, development indicated a weak to strong positive reaction and the result were quantitatively recorded in EL_X 800 ELISA reader at 405 nm.

In all the ELISA experiments conducted, the values were accepted as positive when the 405 nm readings were twice greater than the mean as absorbance of the virus free control sample (healthy tissue) (Hobbs *et al*., 1987).

3.2.2 RT-PCR

3.2.2.1 Plant material used to isolate GBNV

GBNV infected tomato leaf samples were collected from fields infected with GBNV in the University of Agricultural Sciences Campus Dharwad, Karnataka.

RNA Isolation

Eukaryotic mini RNA isolation kit and RT- PCR kits of Eppendorf were used. Diethylpyrocarbonate (DEPC) 0.1 per cent, β-merceptoethanol (14.3M), and absolute ethanol were from Sd Fine Chemicals, Mumbai and the components were from Hi Media, Mumbai. All the solutions and glassware were treated with 0.1 per cent DEPC water and used after sterilization. RNA isolation and RT-PCR reaction was done as prescribed for the respective kits.

The total RNA was isolated from infected and non-infected leaves of tomato using Eppendorf Perfect RNA^{TM} and Eukaryotic Mini RNA Isolation kit. Accordingly, one gram of each sample was ground in liquid nitrogen and about 100 mg of the ground material was taken in a tube containing 350µl lysis solution and homogenized. The homogenized sample was transferred to a 1.5 ml microcentrifuge tube and spun at 15,700 rcf for five minutes. The supernatant was transferred to a fresh 1.5 ml tube, to which 350 µl of 70 per cent ethanol was added and mixed by gentle inversions. To this mix, 200 µl of Perfect Binding Matrix Solution was added and mixed gently, the lysate/ Binding Matrix mixture was pepited into a Perfect RNA Binding Matrix Spin Column and centrifuged at 15,700 rcf for 30 seconds. Two successive washes with 700µl wash solution 1 and 500µl wash solution 2 were given by centrifugation for 30 seconds at 15,700 rcf, before eluting RNA from the column with RNase free water. About 50µl of RNA preparation was collected and stored at -20^{0}C.

REVERSE TRANSCRIPTION

cDNA was synthesized from mRNA through reverse transcriptase in a 20 µl reaction mixture containing 5 mg of total RNA isolated from the infected and non infected samples of tomato. Eppendorf RT-Kit from Eppendorf was used for RT-PCR.

Reverse transcription reaction mixture per tube

The following components were added to prepare reaction mixture in the order mentioned below.

Components	Concentration	Volume (µl)
RT-buffer	10×	2
dNTPs	10 mM each	2
Oligo-dT	50 ng/µl	2
RNAs inhibitors	100 U/ml	1
Template	2.50mg/ µl	1
RT-enzyme	15 U/µl	1
Sterile nano pure water	-	11
Total	-	20

Protocol

1) All the reactions were set up on ice to avoid premature cDNA synthesis and minimize the risk of RNA degradation.

2) Two tube RT-PCR was performed. The reverse transcription reaction and PCR was performed sequentially in two separate reaction tubes.

3) Soon after adding the template RNA to reaction mixture, it was mixed and vortexed for about 5 seconds and briefly centrifuged.

4) The reverse transcription conditions employed for the reverse transcription of GBNV coat protein mRNA were;

Temperature (℃)	Time (mins)
65	5
42	60
4	Hold

AMPLIFICATION GBNV PARTIAL COAT PROTEIN SEQUENCE

Primers specific for the GBNV partial coat protein sequence

The nucleotide sequence and the amino acid of coat protein gene of Peanut bud necrosis virus (GBNV) was downloaded from NCBI database. The gene sequence from different hosts were multialigned using CLUSTALX and the degenerate PCR primers were designed to amplify ~830 bp coat protein gene sequence using GENETOOL software.

Primer for cloning of GBNV CP gene

Degenerate F	5' ATGTCTAMCGTYAAGCAVCTHAMCG 3'
Degenerate R	5' TTACAMTTCCARMGAAGKRCHAG 3'

M = A/C R = A/G

Y = C/T K = G/T

H = A/C/T V = A/G/C

Standardization of PCR Conditions

PCR conditions were standardized to amplify a single sharp amplicon by varying primer concentration, primer annealing temperature and the concentration of dNTPs. Different primer concentrations such as 2.5 pM, 5 pM, 10 pM were used for the purpose. PCR was performed for each set of primers using Eppendorf Master Cycler (5331) to optimize reaction conditions. Based on this exercise, the following reaction mixture was employed for further studies.

Reaction mixture

The master mix required was prepared from components obtained from Bangalore Genei, Bangalore and Eppendorf, Germany and was distributed into 0.2 ml PCR tubes and volume was made up to 20 µl with sterile nano pure water.

Components	Concentration per µl	µl per reaction
Template cDNA	100 ng	1
Primers	5p M each	1 each
Taq DNA Polymerase	3U	0.33
Assay Buffer	10X	2
dNTPs	1mM each	2

$MgCl_2$	25 mM	0.5
Sterile H_20	-	12.2
Total	-	20

3.2.2.2 PCR Amplification

The following PCR amplification conditions were employed for amplification of GBNV-coat protein gene partial sequence.

Stage	Step	Temp °C	Duration (min)	No.of cycles
1	Initial denaturation	94	5	1
2	Final denaturation	94	1	39 cycles
3	Annealing	52	1	
4	Extension	72	2	
5	Final extension	72	20	1
6	Hold	4	Hold	

Electrophoresis

About 20 µl of the reaction mixture from each tube with 3 µl of loading dye were loaded onto 1.2 per cent agarose gel along side 100 base pair DNA ladder as molecular weight marker. Electrophoresis was done at 50 V for initial 30 min and then 70 V for 1 hr. The buffer used was 1x TAE at pH=8.0. The DNA bands in the gel were visualized on a UV-transilluminator and documented using a gel documentation system (UviTec Cambridge, England).

PCR BASED CLONING

Cloning of GBNV partial coat protein (CP) with both sets of primers was done in vector pTZ57R/T by using InstT/A cloning kit (MBI Fermantas USA) following the procedure outlined in the user's manual at IABT (MB_4) UAS, Dharwad.

Gel elution of the PCR amplicon

The specific amplicon of about ~830 bp in CP was amplified by giving 45 minutes of final extension in PCR reaction (as per user's manual) and run on a 1 per cent agarose gel. The specific amplicons were cut using a sharp sterile scalpel by keeping the gel at low intensity UV transilluminator and collected in sterile pre-weighed 2.0ml microcentrifuge tubes. The Eppendorf gel cleanup kit was used to elute the amplicons from the agarose block as described in user's manual.

3.2.2.3 Cloning of PCR product

The purified PCR fragment of about 830 bp was separately ligated to pTZ57R/T cloning vector (2886 bp) as described in InsT/A clone™ PCR product cloning kit (K1214) MBI, Fermentas, USA.

Ligation

For ligation, an optimal molar ratio of ends of vector: insert (1:3) was computed and the components of ligation mix were added to 0.5 ml micro centrifuge tubes and was incubated at 16°C for 16 hr.

Components	Coat protein (µl)
Plasmid vector pTZ57R/T DNA (0.055µg/µl)	3.0 aprox. (0.165µg)
Purified PCR fragment	4.0 (aprox. 0.54 pmol ends)
10x ligation buffer	3.0
PEG 4000 solution	3.0
Sterile water	16.0
T4 DNA ligase, 5U	1.0
Total	30

Preparation of competent cells

E. coli DH5α competent cells were prepared by following the protocol mentioned in Sambrook and Russell (2001) with minor modifications under aseptic conditions. An isolated colony from *E. coli* DH5 α plate was inoculated in 5 ml Luria broth and incubated at 37°C overnight at 200 rpm. Next day, the culture was diluted to 1:100 using Luria broth *i.e.*, 0.5 ml of culture was added to 50 ml of Luria broth. It was incubated for 2 to 3 hours till an OD of 0.3 to 0.4 at 600 nm was attained. The culture was chilled in ice for 30 min and 25 ml of culture was dispensed into two sterile centrifuge tubes. The cells were pelleted at 7300 rcf for 5 min at 4^0C, the supernatant discarded and the pellet was suspended in 12.5 ml ice-cold 0.1 M $CaCl_2$. The centrifuge tubes were again kept in ice for 30 min, and later centrifuged at 4800 rcf for 5 min. The pellet so obtained was dispensed in 1 ml of ice-cold 0.1 M $CaCl_2$ and 88 µl of dimethyl sulfoxide (DMSO) was added. About 100 µl of the competent cells were distributed to pre-chilled 1.5 ml micro centrifuge tubes and stored at $–20^0$C or used immediately for transformation.

3.2.2.4 Transformation into *E. coli* DH5α

About 100 µl of freshly prepared competent cells were taken in a chilled micro centrifuge tube and 10 µl of ligation mixture was added, mixed gently and chilled on ice for 30 min. Heat shock was given by shifting the chilled mixture to 42°C water bath for exactly 2 min, and immediately chilled in ice for 5 minutes. To this, 900 µl of Luria broth was added and incubated at 37°C at 200 rpm for 45 minutes to allow bacteria to recover and express the antibiotic marker encoded by the plasmid. The cells were pelleted at 15,700 rcf for 1 min, 900 µl of supernatant discarded and the pellet was dissolved in the remaining 100 µl of supernatant and spread on Luria agar plates having Amp_{50}, X-gal, IPTG and incubated overnight at 37°C.

The recombinant clones were identified by blue/white assay. After incubation, only white colonies having recombinant vectors were picked up and streaked on plates having Luria agar with Amp_{100}, X-gal, IPTG and incubated at 37°C overnight, for multiplication.

3.2.2.5 Confirmation of clones

The confirmation for the presence of desired DNA fragment in cloning vector was done by PCR amplification using specific primers and by restriction analysis.

For PCR confirmation of clones, the template DNA from plasmid was isolated following the alkaline lysis protocol of Brimion and Dolly (1979). White colonies were inoculated to 10 ml Luria broth with ampicillin (100 µg/ml) overnight at 37°C over shaker at 175 rpm. Overnight grown culture was centrifuged at 15,700 rcf for 2 min at 4°C in 2.0 ml microcentrifuge tubes. The supernatant was removed and pellet was washed with STET (0.25 volume of original culture). It was centrifuged at 15,700 rcf for 2 min. The pellet was resuspended in 200 µl of ice-cold alkaline lysis solution I by vigorous vortexing. Later, 400 µl of freshly prepared alkaline lysis solution II was added to each tube and the contents were

mixed by inverting the tubes 4 to 5 times and kept in ice for about 5 min. To this suspension, 300 µl of alkaline lysis solution III was added and again mixed thoroughly by gently inverting the tubes 4-5 times. The tubes were stored on ice for 5 minutes and centrifuged at 15,700 rcf for 8 min. The supernatant was transferred to fresh tubes and equal volume of phenol: chloroform isoamyl alcohol (25:24:1) was added to precipitate proteins and was mixed well. It was centrifuged at 15,700 rcf for 10 min at 4°C. The aqueous layer was transferred to a fresh tube and two volumes of isopropanol were added. The contents were mixed and allowed to stand for 2 minutes at room temperature. The solution was later centrifuged at 15,700 rcf for 5 min. The supernatant was discarded and pellet was washed with 70 per cent ethanol and spun for 1 min at 15,700 rcf to recover the plasmid. The supernatant was discarded pellet dried completely was dispensed in 25 µl of $T_{10}E_1$ (pH=8.0) containing 3 µl of RNase A (10 mg/ml). The solution was kept at 50°C for 15 min and then stored at -20°C.The plasmid DNA was visualized on 0.8 per cent agarose gel as described, using *HindIII/EcoRI* double digest, as DNA marker.

Confirmation of the presence of cloned fragment was done by PCR amplification of clones with respective primer pairs

3.2.2.6 Sequencing of clones

The partial coat protein gene cloned in pTZ57R/T was sequenced using M13F/R primers employing primer walking technique at Bangalore Genei Private Ltd., Bangalore. The sequence results were analyzed using BLAST algorithm available at http://www.ncbi.nlm.nih.gov.

3.2.3. Electron microscopy

To examine the virus from purified preparations in the electron microscope.

Partially purification procedure

Young Tomato leaves showing primary symptoms were collected and triturated in chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.01 M Na_2SO_3 at the rate of 3 or 4ml buffer for each gram of tissue. Then filtered through two thicknesses of cheese-cloth. Clarified at 5000 rev min^{-1} (rpm) for 5 min in a refritertaed centrifuge at 4°C and to the supernatant, NaCl added to give 0.2 molarity and polyethylene glycol ([(PEG) mol. Wt. 6000-8000] to give 4 per cent. After dissolving NaCl and PEG, mixture was allowed for 1.5 to 2 h at 4°C. Then precipitate was collected by centrifuging at 10000 rev min^{-1} for 10 min in a refrigerated centrifuge at 4°C then resuspend the precipitate in 0.01 M potassium phosphate buffer, pH 7.5, containing 0.1 M sodium sulfite (0.01 PPBS). Finally clarified at 5000 rev min^{-1} for 10 min in a refrigerated centrifuge at 4°C and this was used as a partially purified sample.

A drop of partially purified virus preparation was placed on the carbon coated grids and allowed to settle for 2-3 minutes. The excess of sample was removed by blotting paper. A small drop of 2 % uranyl acetate (pH 7.0) was placed on it and allowed to react for 2-3 minutes. The excess stain was drained off by touching a blotting paper strip to the edge of the grid. The grids were dried for 15 to 30 min and examined under JEOL100 X transmission electron microscope (TEM) 24000 x magnifications at Indian Institute of Horticulture Research (IIHR) Hesaragatta, Bangalore.

3.3 TRANSMISSION STUDIES

3.3.1. Mechanical transmission

For mechanical transmission, inoculum was prepared in 0.05 M potassium phosphate buffer (pH 7.0) consisting of virus infected tomato leaves and buffer in the ratio of 1:2 (W/V). The extraction was done using ice chilled buffer, pestle and mortar and passed through a muslin cloth. Fifteen to twenty five days old tomato seedlings were inoculated with plant extracts by gentle smearing on the upper surface of leaves with the inoculum containing carborendum (600 mesh sizes) and allow them to air dry for five minutes. After inoculation, leaves of the plants were washed with tap water.

Test plants used for mechanical inoculation were raised from healthy seeds in an earthen pots containing steam sterilized soil, sand and compost (2:1:1) (V/V) mixture and maintained in an insect free glass house for 4 to 5 weeks. Observations were recorded on symptom development. The virus inoculum was maintained on cowpea (cv. C-152).

3.3.2. Insect transmission

3.3.2.1 Collection and maintenance of thrips colony

Thrips palmi, adults were collected from watermelon field Saidapur farm. They were transferred to a glass vial and immobilized by placing them in a refrigerator for 15 minutes. They were transferred to a petridish floating on ice water different species were sorted out under a stereoscopic binocular microscope. Identification was based on the characters given in the publication by Reddy *et al.,* (1990).

3.3.2.2. Rearing of thrips

Thrips were reared on both groundnut leaflets and on bean pods as described below.

3.3.2.3 Maintenance of thrips on peanut leaflets

Thrips were reared on groundnut leaflets using the method reported by Amin *et al.* (1981) *Thrips palmi* Colonies were successfully reared on detached groundnut leaflets.

Identification characteristics of *Thrips palmi* and *Scirtothrips dorsalis*

Characteristics	*Thrips palmi*	*Scirtothrips dorsalis*
Adult female colour and length	Adult female straw yellow to pale brown, 0.9mm long	Adult female relatively small, yellow in colour 0.7 mm long.
Pronotum	Pronotum having 2 pairs of setae on the posteriolateral margin, no setae on the anteriolateral margin	No setae on the pronotum. dark patches on the dorsal side of abdominal tergites.
Wing characteristics	Fore wings with broken row of wing vein setae	Fore wings with few small setae on the vein, hind wing with 2 setae.
Features of larvae	Larvae pale yellow, move slowly and bend their abdomen while changing the direction	Larvae whitish both Larvae and adults actively moving in a darted fashion.

Under controlled conditions in glass vials (3x1inch) leaf lets remained in good condition for at least 10 days. Facilitating oviposition and larval emergence the vials before were washed with water and sterilized at 160^0C for 1hour immobilized thrips were released into a glass vial, and which was held in an inverted position thrips moved up ward and gathered at the upper portion of the inverted vial. Immediately a young leaf let of groundnut was introduced into the vial and then closed with a cork. After allowing, one day for oviposition, the thrips were dislodged from the leaflet onto a paper by tapping the inverted vial and the dislodged ones were collected into a separate vial, later fresh leaflets were introduced into the vial for further egg laying. This process was continued for about 10 days during which 90 per cent of the total egg laying was completed. The leaflets with eggs were transferred to a new vial on alternate days.

First instars of *Scirtothrips dorsalis* were directly collected from the rose plants and used for transmission studies.

3.3.2.4 Maintenance of thrips on French bean pods (*Phaseolos vulgaris* L)

Virus free *T. palmi* was raised on French bean pods (*Phaseolus vulgaris* L.) at 25 to 28℃ ± 0.5℃ and 8 hr day length .The colony was started with the adults collected from watermelon plants. To obtain uniformly aged larvae, fresh bean pods were placed in the thrips colonies for oviposition. After 24 hrs beans were taken from the colonies and removed infecting thrips, cleaned and placed separately at 25℃. Larvae of 12 hr old emerging from bean pods were used for the experiments.

3.3.2.5 Acquisition Access Period (AAP)

Young tomato leaves showing primary symptoms were kept in the petriplate along the rim of the petriplate a thin layer of water is poured and about 30 to 40 first instar larvae were released on tomato leaves showing chlorotic ring spots using a fine moist camel hair brush. The first instar larvae were allowed for AAP of 4 days at room temperature. Larvae feeding on healthy leaves served as check. The larvae were transferred to each glass vial containing healthy groundnut leaf lets. Adults emerged in 7 to 8 days were used in transmission studies.

3.3.2.6 Inoculation Access Period (IAP)

Fifteen to twenty larvae of *Thrips palmi* (Karny) placed with a fine hair brush either singly or in pairs on seedlings of tomato plants (cv. Megha) in cylindrical plastic cage. Thrips were observed for normal movement to ensure that injury had not occurred during transfer. The plants were observed for 20 days to express symptoms expression.

3.4 HOST RANGE STUDIES

Plant species belonging to different families *viz*., Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, and Solanaceae were used for host range studies.

Test plants were raised from healthy seeds in earthen pots containing the mixture of steam-sterilized soil, sand and compost in 2:1:1 ratio. (V/V), and maintained in an insect free glass house. Five plants of each test species were inoculated with the sap extracted from virus infected tomato leaves by conventional leaf rub method. All the leguminous plants were inoculated on primary leaves before the emergence of first trifoliate leaf. All other plants were inoculated on the first leaf and fully expanded leaves. The inoculated host plants were kept for observations for about 4 to 6 weeks were back index on assay hosts. *Vigna unguiculata* cv. C-152 and *D. stramonium* for the detection of latent infection if any. The host range studies were conducted by raising seedlings of various plant species of tobacco and chenopodium were inoculated at 6-8 leaf stage, where as cucurbits and legumes were inoculated at primary leaf stage and others at 2-4 leaf stage different host plants were mechanically inoculated and observed for 30-40 days in glass house. All inoculated plants were checked for virus by assay on cowpea, *D. stramonium* and using ELISA.

3.5. MANAGEMENT OF BUD BLIGHT DISEASE

3.5.1. Reaction of tomato cultivars/hybrids against GBNV under field condition

Totally 22 tomato cultivars and commercial varieties namely Marikrit, NS-2535, PKM-1, Vybhav, Nandi, NS-816, NS-2532, Megha, NS-564, Tomato hybrid -530, NS -53, Sankranti, NS-658, NS-719 (519), Sonam, NS-585, NS-812, Malini, Utsav, Ns-563, Arka Vikas,, VC-2048 were sown in nursery beds and transplanted in the main field 24 days after sowing. Each variety, commercial hybrid was planted in 2 rows of 6 meter length in summer of 2007-08. Time of first symptom appearance and vector population was recorded in each line and incidence of both was recorded up to twelve weeks after planting.

The following scale (Joi and Summanvar, 1989) was employed for scoring the disease reaction.

Reaction categories

Grade	PDI	Reaction Category	Symptoms
A	0.00	Immune (I)	No symptoms
B	0.1 to 5.00	Resistant (R)	Initial symptoms on younger leaf lets
C	5.1 to 10.00	Moderately resistant (MR)	Symptoms extended up to petioles
D	10.1 to 15.0	Moderately susceptible (MS)	Necrosis of growing branch including buds
E	15.1 to 25.0	Susceptible	Necrosis extended up to stem, covering all plants.
F	25.1 and above	Highly susceptible	Severe necrosis and wilting.

The time taken for first symptom appearance after transplanting was recorded in different tomato cultivars.

Days taken for first appearance of symptoms after transplanting	Characteristics
10-20	Very early (VE)
21-30	Early
31-40	Moderately late (ML)
41-50	Late (L)
51 and above	Very late (VL)

This experiment was conducted in the field to know the source of resistance.

3.5.2 Induction of antiviral principles (AVP) through application of leaf extracts (10%)

Sorghum and coconut leaves free of any visible symptoms of diseases were collected, and air dried till constant weight was reached. The leaf material after removal of midrib was powdered. The powdered leaf material was immersed in water at 50-60°C for one hour. The final volume was maintained at 1:9 (w/v) for leaf powder to water. The extract was filtered through cotton wool.

The leaf extracts were sprayed 24 hrs prior to virus inoculation, and 48 hours after inoculum application for each treatment. A treatment with application of GBNV inoculum alone was also included. Cowpea plants were used for this experiment.

Experimental details:

T_1	T_2	T_3	T_4	T_5
Spray leaf extract ↓ after 24 hrs inoculation ↓ observed for symptoms development	First inoculation ↓ after 48 hrs spray leaf extract ↓ observed for symptoms development	First inoculation ↓ after 48 hrs spray leaf extract ↓ after 24 hrs inoculation ↓ observed for symptoms development	Inoculation ↓ No spray ↓ observed for symptoms development	Control ↓ No spray No inoculation ↓ observed for symptoms development

3.5.2.1. Activity of antiviral principles from plant products

The resistance activity of AVP from sorghum (*Sorghum bicolor* L.) and coconut (*Cocos nucifera* L.) were tested. The AVPs from leaves of sorghum and coconut were obtained following procedure described by Narayanaswamy and Ramaiah (1983).

The activities of AVP from the plant products were tested by spraying the AVP solution on the primary leaves of cowpea at the rate of 1 ml per plant using a micro glass applicator. The primary leaves of cowpea were inoculated with virus inoculum prepared from cowpea leaf tissue showing 10 local lesions in 1ml of 0.05 M potassium phosphate buffer (pH 7.0) containing sodium sulphite, after different periods of application of AVP. These leaves were further used for isozyme studies.

3.5.2.2. Estimation of Antiviral principles

To know the resistance mechanism, the activity of different enzymes like viz. peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) were estimated. The susceptible genotype cv. C-152 of cowpea were used in this study, cowpea plants were maintained in plastic pots and pots were kept in glass house. The inoculated seedling were covered with nylon cloth to prevent other insect vectors.

3.5.2.3. Enzyme extraction

The sampling was done 10 days after symptom expression. The cowpea leaf samples showing chlorotic lesions were collected from inoculated and control plants placed in the ice box containing ice cubes and brought to the laboratory and immediately homogenized with liquid nitrogen. One gram of powdered leaf sample was extracted with 2 ml of 0.1 M sodium phosphate buffer (pH-7.0) at 4°C. The homogenate was centrifuged for 20 minutes at 10,000 rpm.

3.5.2.4. Assay of phenylalanine ammonia lyase (PAL)

One gram of each treatment plant sample was homogenized in 2ml of ice cold 0.1 M sodium borate buffer, pH 7.0, containing 1.4 mM of 2-mercaptoethanol and 50 mg of insoluble polyvinyl pyrrolidone (PVP). The resulting extract was filtered through cheese cloth and the filtrate was centrifuged at 20000 rpm for 15 min at 4°C and the supernatant was used as the enzyme source. PAL activity was determined as the rate of conversion of L-phenylanine to trans-cinnamic acid at 290 nm. Enzyme activity was expressed in fresh weight basis as nmol transcinnamic acid mg/min/gm of sample (Nanodrop, spectrophotometer ND1000).

3.5.2.5. Assay of peroxidase (PO)

Assay of PO activity was carried out as per the procedure described by Hammerschmidt *et al.* (1982). The reaction mixture consists of 2.5ml of a mixture containing 0.25 per cent (v/v) guaiacol in 0.01 m sodium phosphate buffer pH 6.0 and 0.1m hydrogen peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction which was followed colorimetrically at 470 nm. Crude enzyme preparations were diluted to give changes in absorbance at 470nm of 0.1 to 0.2 absorbance units/min. Activity was expressed as the increase in absorbance at 470nm min^{-1} mg^{-1} g^{-1} of sample.

3.5.2.6. Assay of polyphenol oxidase (PPO)

One gram of each treatment sample was homogenized in 2ml of 0.1 M sodium phosphate buffer (pH 6.5) at 4℃. The homogenate was centrifuged at 20000 rpm for 15 min at 4℃. The supernatant served as enzyme source and polyphenol oxidase activity was determined as per the procedure given by Mayer *et al.* (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract which was followed colorimetrically at 470 nm. To start the reaction, 200 µ1of 0.01M catechol was added and the activity was expressed as change in absorbance min^{-1} mg^{-1} g^{-1} of sample.

3.5.3 Management of bud blight disease of tomato

An experiment was conducted to know the effect of different treatments in nursery and field spray on disease development in the main field.

Details of experiment:

Treatments	10
Replications	3
Plot size	3.6 mts. X 4.5 mts.
Design	RCBD
Cultivar	Megha
Spacing	60 cm x 45 cm

Treatment details:

T_1 - Seed treatments with Imidachloprid (0.005%)

T_2 - Border crops with maize sown 15 days before transplanting (cv. South African tall).

T_3 - T_1 + 4 sprays of sorghum and coconut leaf extract (10%) at 15 days interval after transplanting alternatively

T_4 - T_2 + T_3

T_5 - T_1 + Thiomethoxom (0.05%)- 4 sprays at 15 days interval after transplanting.

T_6 - T_1 + I^{st} spray of Intrepid (0.5%) + 2^{nd} spray of Acetamiprid (0.02%) + 3^{rd} spray of Thiomethoxom(0.05%) at 20 days interval after transplanting.

T_7 - 8% alcohol-4 spray at 20 days intervals after transplantings

T_8 - Imidachloprid (0.005%)- 4 sprays at 15 days interval after transplanting

T_9 - Control (No spray)

T_{10} - Package of practice

A completely randomized replicated field trail was conducted to know the effect of different treatments on the incidence and thrips population on tomato.

Preparation of leaf extract

The leaves of coconut and sorghum leaves were brought to laboratory, after weighing 500gm of leaf material, washed the material thoroughly to remove the dust particles if any. Then the leafy materials were macerated in a grinder, mixed using water 1:9 (w/v) ratio and the mixture was squeezed by using a two layered muslin cloth and the filtrate was diluted at 1:10 and used for field spray.

4. EXPERIMENTAL RESULTS

4.1 SYMPTOMATOLOGY

In the field, the symptoms of GBNV on tomato cv. Megha showed yellowing, chlorotic, and necrotic ring spots on leaves and tip necrosis and die back were major symptoms followed by stunted growth. Another conspicuous symptom was the presence of longitudinal, brown, necrotic streaks on the petioles, stem and fruit stalk. Fruit set was markedly reduced. Further characteristic chlorotic ring spots were often observed on red ripe tomatoes (Plate 1).

In the glasshouse, tomato cv. Megha plants inoculated with GBNV virus showed chlorotic and necrotic ring spots on inoculated leaves followed by yellowing, reduction in leaf size and also necrosis and dieback of growing tip. Infected plant became stunted and died after some days. These symptoms resembled similar to the symptoms observed in the infected tomato plants under field conditions. The GBNV collected from the infected tomato under greenhouse condition was inoculated to cowpea cv. C-152 and *Datura stramonium*, these hosts showed local lesions, indicating that the virus is the causal agent of the disease (Plate 1). This virus was also confirmed through ELISA using polyclonal antibodies of PBNV.

4.2 SURVEY FOR THE GROUNDNUT BUD NECROSIS TOSPOVIRUS ON TOMATO

The field surveys were undertaken to assess the bud blight disease caused by the GBNV in major tomato growing regions of Dharwad, Belgaum, Haveri and Kolar districts during summer months from February to May 2008.

From the survey it is revealed that the disease was severe in all the districts during February-May 2008 and disease incidence ranged from 12.5 to 94.4 per cent in different parts of the districts surveyed. The highest incidence (94.4%) of bud blight was noticed in fields of Kyalanur village in Kolar district, whereas least (12.5%) incidence of the disease was recorded at Guledkoppa village of Dharwad district. The village wise disease incidence has been presented in Table 1.

The highest average disease incidence was recorded in Kolar district (64.85%) followed by Haveri (60.69%), Dharwad (57.90%) districts and least incidence was noticed in Belgaum (50.00%) district.

The highest disease incidence of bud blight was observed at Nesargi (87.5%) of Gokak taluk of Belgaum district, whereas least incidence was observed at Hirebagewadi (22.5%) in Belgaum taluk. In Dharwad district, highest incidence was recorded at Aminbavi (88.5%) in Dharwad taluk. Whereas, least incidence was observed at Guledkoppa (12.5%) in Dharwad taluk. In Haveri district the highest disease incidence was observed at Mannur (80%) in Savanur taluk, whereas lowest incidence was recorded (28%) at Sudambi in Byadagi taluk. In Kolar district, the highest disease incidence was recorded at Kyalanur (94.4%) of Kolar taluk. Whereas, least incidence (20%) observed at Odagur in Kolar taluk. District wise incidence has been presented in Table 2 and graphically represented in Fig. 1.

With respect to varieties, the per cent incidence was highest on Abhinav (94.4%) in Kolar district and least incidence (12.5%) was recorded on Utsav in Dharwad district. The incidence of bud blight disease on different varieties are presented in the Table 3.

4.3 DIAGNOSIS OF DISEASE

4.3.1 By ELISA (Enzyme Linked Immuno Sorbent Assay)

The GBNV was detected using DAC-ELISA on different hosts which was mechanically inoculated *i.e. Amaranthus retroflexus*, *Emelia sonchifolia*, *Vigna unguiculata*, *Chenopodium album*, *Nicotiana tabacum*, *Solanum lycopersicum*, *Cassia* spp., *Datura stramonium*, *Capsicum annuum, Alternanthera sessiles*. Healthy and buffer were used as

a. Necrotic ring spots on tomato fruit

b. Necrotic streaks on stem

c. Chlorotic spots on fruit

d. Chlorotic lesions on cowpea

e. Chlorotic lesions on datura

Plate 1. Symptoms of GBNY on tomato, cowpea and datura

Table 1. Incidence of bud blight disease in tomato during summer 2008

Sl. No.	District	Taluk	Village/Place	Area (acres)	Variety/ Hybrid	Crop stage	Incidence (%)	Symptoms recorded	Insects noticed
1.	Dharwad	Dharwad	Singanalli	1.0	Mahyco hybrid	Fruit Setting	40	Severe stunting, tip necrosis, necrotic ring spots on leaves small size fruits.	TH,WF
2.			Guledkoppa	0.50	Utsav	Flower Initiation	12.5	Stunting, chlorotic local lesions, necrotic streaks on stems	WF,TH
3.			UAS, Campus	0.10	Megha	Fruit Setting	30	Very clear chlorotic and necrotic local lesion, stunted plants, blighting of flower buds	TH,WF, mites
4.			Gamanaghatti	0.75	Local	Fruiting stage	85.7	Severe stunting, flower bud blight, necrosis on stem chlorotic and necrotic spots on leaves.	TH,WF and LH
5.			Unakal	1.25	Local	Fruiting Stage	34	Severe stunting, flower bud blight, necrosis on stem, chlorotic and necrotic spots on leaves and small sized fruits	TH, WF mites
6.			Aminbhavi	0.50	Local	Fruiting Stage	88.5	Severe stunting, flower bud blight, necrosis on stem chlorotic and necrotic spots on leaves. And pale and yellow necrotic ring spots on fruits.	TH, WF LH, and mites
7.			Shivalli	1.25	Local	Fruiting Stage	81.25	Severe stunting, flower bud blight, necrosis on stem chlorotic and necrotic spots on leaves.	TH, WF
8.			Somapura	0.50	Local	Fruiting Stage	75	Very severe stunting, necrotic rings on fruits and necrosis on stem, fruits are malformed.	TH,WF + LH
9.			Harobelavadi	0.5	Sungro hybrid	Fruit formation	60	Necrosis on flower buds necrotic ring spots on fruits. Leaves, necrotic streaks on stem	TH, WF and mites
10.	Dharwad	Hubli	Hebsur	0.5	Local	Flower initiation	60	Stunted growth very severe, spots on leaves, bud blight and stem necrosis	TH, WF
11.			Annigere	0.5	Local	Flower intitiation	80	Stunted growth very severe, spots on leaves, bud blight and stem necrosis and wiliting	TH, WF
12.			Bhadrapoora	0.25	Local	Flower initiation	40	Leaf bronzing, chlorotic local lesion, chloratic rings, and top necrosis	TH, WF
13.		Kundagol	Nalavadi	1.0	PKM -1	Fruit setting	66.6	Leaf bronzing, chlorotic local lesion, chlorotic rings, and top necrosis stunted plants with, bud blighting, stem necrosis	TH, WF and LH
14.			Devanuru	1.0	PKM -1	Fruiting Stage	60	Extreme stunting, stem necrosis, necrotic spots on leaves, ring spots on fruits.	TH, WF and mites
15.			Kambadhalli	0.5	PKM-1	Fruiting stage	60	Severe stunting, chlorotic and necrotic ring spots on leaves, ring spots on young fruits.	TH, WF

Table 1. Contd……

16.		Navalagunda	Belavatagi	1.0	Local IR	Flower initiaion	70	Stunted plants, leaf bronzing, chlorotic ringspots on leaves, bud blight, necrotic streaks on stem.	TH, WF and LH
17.	Dharwad		Amargol	0.5	PKM-1	Flower initiation	60	Stunted plant, leaf bronzing chlorotic ringspots on leaves	TH, WF and LH
18.			Karalawada	1.25	Megha	Flower initiation	55.5	Severe stunting on some of the infected plants, Chlorotic and necrotic ring spots on leaves, blighting of flower buds, stem necrosis.	Th, WF and LH
19.			Karalgund	0.5	Local	Flower initiation	64	Severe stunting of some of the infected plants. Chlorotic and necrotic ring spots on leaves, blighting of flower buds, stem necrosis.	TH, WF
20.		Kalagatagi	Hulikoppa	1.0	Sungro hybrid	Fruit Setting	50	Leaf bronzing, stunting, stem necrosis, top necrosis, chlorotic and necrotic local lesion on leaves small sized fruits	TH, WF and LF
21.			Hirenalli	0.5	PKM-1	Fruiting stagbe	42.8	Very clear chlorotic and necrotic local lesion on leaves, flower bud blight, stem necrosis	TH,WF
22.	Belgaum	Belgaum	Hirebagewadi	1.2	Utsav	Fruiting stage	22.5	Severe stunting, necrotic local lesions on leaves stem necrosis and ring spots on fruits.	TH,WF mites
23.			kamatarhatti	0.75	NS-2535	Flower initiation	32	Bronzing of leaves, bud blight, chlorotic and necrotic rings on leaves and stem necrosis	TH,WF +mites
24.		Bylahongal	Sheegehalli	1.00	Mahyco hybrid	Fruiting stage	50	Severe stunting, and wilting of plant, no fruits, bud blighting bronzing of leaves necrosis	TH, WF
25.			Banasamuddi	0.6	Local	Fruit setting	50	Leaf bronzing, chlorotic local and necrotic local lesions, stem necrosis stunted plant small sized fruits.	WF,TH LH
26.			Giriyela	0.25	Utsav	Fruit Setting	58.3	Leaf bronzing, chlorotic local and necrotic local lesions, stem necrosis stunted plant small sized fruits.	TH,WF and mites
27.		Belgaum	M.K. Hubli	0.5	Ashoka Seeds	Flower setting	77.5	Leaf bronzing, chlorotic local and necrotic local lesions, stem necrosis stunted plant small sized fruits.	TH, WF and LH
28.		Gokak	Banachina maradi	1.0	Mahyco hybrids	Flower initiation	40	Stunted plant, leaf bronzing, chlorotic and necrotic ring spot on leaves, flower bud blighting, necrotic streaks on stem.	TH, LH, and WF
29.			Nesargi	1.0	Seminis hybrid	Fruiting stage	87.5	Extreme stunting, small sized fruits, necrotic local lesions on leaves necrotic streaks on stem ring spots on fruits	TH, WF and mites

Table 1. Contd……

30.			Ankalagi	1.50	Rashmi	Flower setting	55	Severe stunting, chlorotic and necrotic local lesions, necrotic streaks on stem	TH, WF and LH
31.		Savadatti	hirehulligere	1.0	Rashmi	Flower initiation	54	Stunted plant, flower bud blighting, chlorotic local lesions on leaves, necrotic streaks on stem	TH, WF LH,
32.			Kabbenuru	0.5	Rashmi	Flower initiation	60	Stunted plant, flower bud blighting, chlorotic local lesions on leaves necrotic streaks on stem	TH,WF and LH
33.			Karikatte	1.0	Local	Flower setting	53.3	Severe stunted plant, necrotic streaks on stem, necrotic local lesions on leaves.	TH, WF and mites
34.			Yadalli	1.25	Rashmi	Fruiting stage	80	Extreme stunting, bronzing of leaves, ring spot on fruits.	TH, WF LH, mites
35.			Inamhongal	1.0	Rashmi	Flower setting	66	Severe stunting of plants, bronzed leaves necrotic streaks on stem.	TH and WF
36.	Haveri	Ranebennur	Itagi	1.25	Sungro seeds	Flower setting	58.3	Severe stunting of plants, bronzed leaves necrotic streaks on stem.	TH,WF mites
37.			Halageri	1.0	Utsav	Flower initiation	53.3	Stunting, chlorotic and necrotic local lesions on leaves leaf bronzing, necrotic streaks on stem.	TH, WF and LH Leaf minor
38.			Taredahalli	1.25	Mahyco seeds	Fruiting stage	70	Severe stunting, bronzed leaves, completewilted plants, ring spots on young and matured fruits.	TH, WF and LH
39.			Ranebennur	1.0	Mahyco seeds	Flower setting	50	Stunting of plants, bronzed leaves, cylindrical necrotic streaks on stem.	TH, WF and mites
40.		Savanur	Savanur	0.75	Mahyco seeds	Flower setting	58.3	Stunting of plants, bronzed leaves, cylindrical necrotic streaks on stem.	TH, WF and mites
41.			Mannur	1.25	Mahyco seeds	Fruiting stage	80	Extreme stunting, bronzing of leaves, ring spots on young and matured fruits, cylindrical necrotic streaks on stem.	TH, WF and mites
42.		Byadagi	Keruradi	0.75	Sungro seeds	Flower initiation	66	Stunted plants, flower bud blighting, chlorotic and necrotic local lesions on leaves, stem necrosis.	TH,LH and WF
43.			Sudambi	1.0	Oriental Seeds	Flower initiation	28	Stunted plants, flower bud blighting, chlorotic and necrotic local lesions on leaves, stem necrosis.	TH,WF
44.		Ranebennur	Gollarahath	0.5	Local	Fruiting stage	77	Stunted plants, leaf bronzing, streaks on stem	TH,WF and Leaf mino
45.			Gutla	0.5	PKM-1	Fruiting stage	66	Stunted plants, leaf bronzing, streaks on stem	TH, WF

Table 1. Contd……

46.	Kolar	Kolar	Bellur	1.0	NS-618	Flower initiation	50	Stunted plant, necrotic streaks on stem, Chlorotic and necrotic local lesions on leaves.	TH,WF and mites
47.			Narasapura	1.5	PKM-1	Fruiting stage	55.7	Extreme stunting, leaf bronzing, ring spots on fruits.	TH, WF and mites
48.			Kamadenahalli	2.0	PKM-1	Fruiting stage	84	Severe stunting, leaf bronzing, necrotic streaks on stem, necrotic local lesions on leaves	TH,WF
49.			Poovandahalli	1.5	Abhinav	Flower initiation	85	Stunted plant, flower bud blighting, chlorotic local lesion on leaves, streaks on stem.	TH,WF
50.			Odagur	2.5	Abhinav	Flower initiation	20	Stunted plant, flower bud blighting, chlorotic local lesion on leaves, streaks on stem.	TH,WF and leaf minor
51.			Kyalanur	0.5	Abhinav	Fruiting stage	94.4	Extreme stunting fully of bronzed leaves in field, very clear ring spots on young and matured fruits.	TH,WF

TH = Thrips, LH = Leaf hopper, WF = White fly

Table 2. Bud blight disease incidence - District wise

Sl. No.	District	Number of fields	Per cent Disease Incidence		
			Minimum	Maximum	Average
1	Belgaum	66	22.5	87.5	50.00
2	Dharwad	105	12.5	88.5	57.90
3	Haveri	50	28	80	60.69
4	Kolar	33	20	94.4	64.85

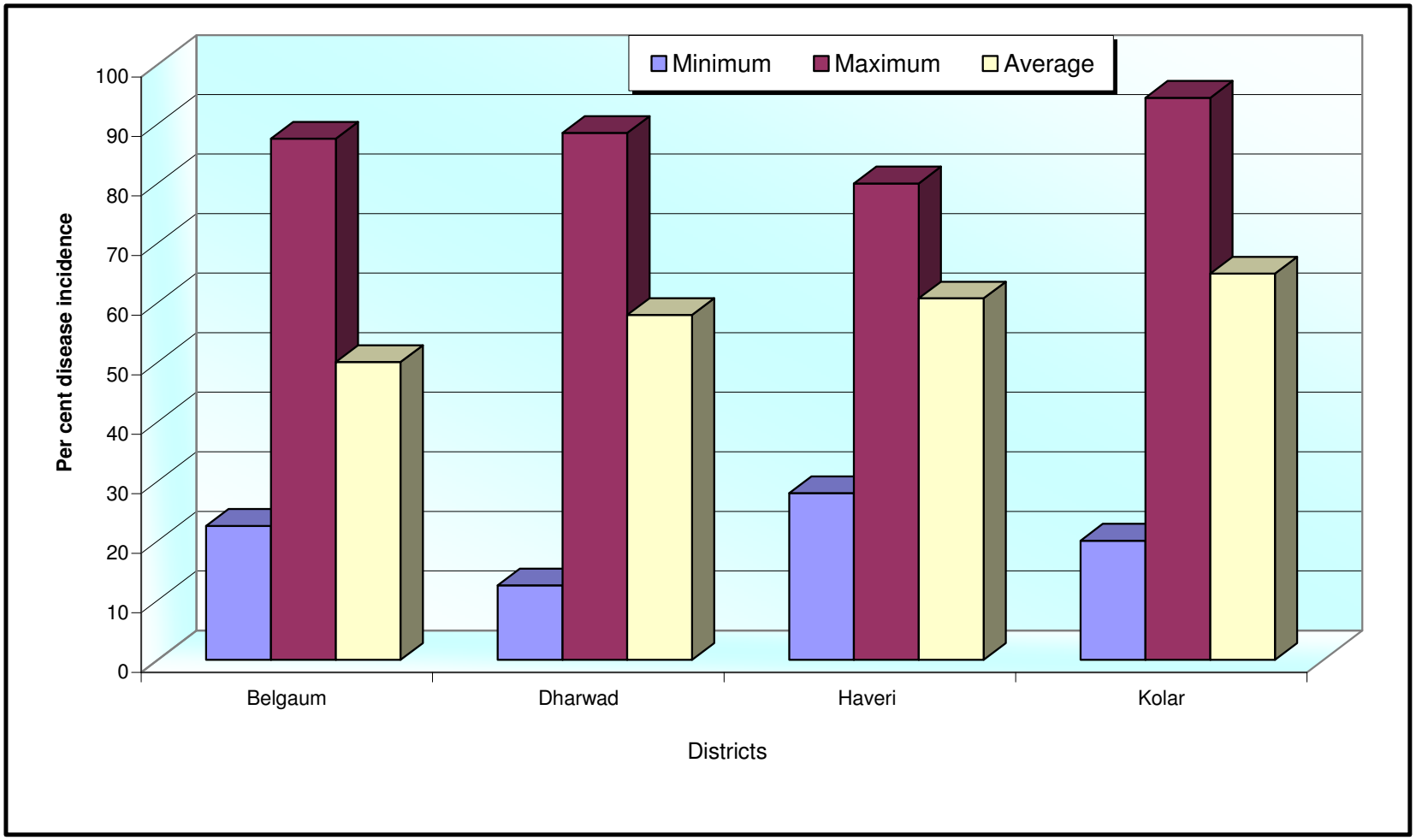


Fig 1. Bud Blight disease incidence – District wise

Table 3. Bud blight disease of tomato - Variety wise

Sl. No.	Variety/Hybrid.	Number of fields	Per cent Disease Incidence Range			
			Belgaum	Dharwad	Haveri	Kolar
1	Mahyco hybrid	35	40-50	40	50-80	--
2	Utsav	20	22.5 -58.3	12.5	53.3	--
3	Megha	10	-	30 - 55.5	-	--
4	Local	60	50-53.3	34 - 64	77	--
5	Rashmi	25	54-80	88	-	--
6	Sungro hybrid	20	--	50 - 60	66	--
7	PKM-1	40	--	42.8-44	66	44-84
8	NS-25 35	2	32	--	--	--
9	Ashoka Seeds	3	77.5	--	--	--
10	Seminis hybrid	5	87.5	--	--	--
11	NS -618	8	--	--	--	50
12	Oriental Seeds	5	--	--	28	--
13	Abhinav	15	--	--	--	20-94.4

--Not grown

Table 4. Detection of GBNV through DAC-ELISA on different hosts using peanut bud necrosis virus (PBNV) polyclonal antibodies

Sl. No.	Hosts	Absorbance at 405 nm
1	*Amaranthus retroflexus*	0.62
2	*Emelia sonchifolia*	0.58
3	*Vigna unguiculata* (Systemic)	1.60
4	*Vigna unguiculata* (Chlorotic)	1.46
5	*Alternanthera sessiles*	0.87
6	*Chenopodium album*	0.56
7	*Nicotiana tabacum*	1.44
8	*Solanum lycopersicum* (cv. Megha)	0.76
9	*Cassia* spp.	0.50
10	*Datura stramonium*	1.46
11	*Capsicum annuum* (cv. Byadagi kaddi)	0.79
12	Healthy control	0.44
13	Buffer control	0.21

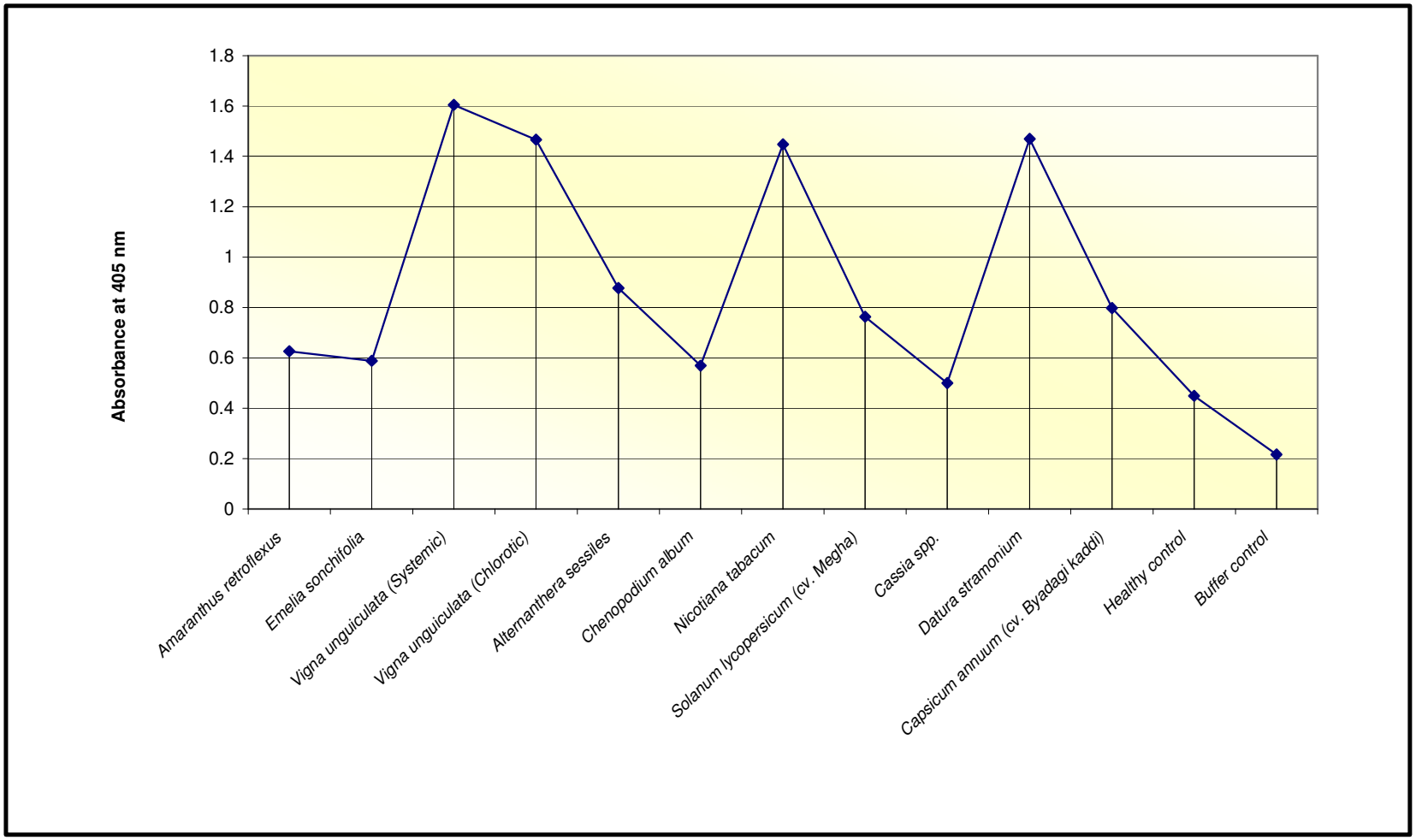


Fig. 2. Detection of GBNV through DAC-ELISA on different hosts using peanut bud necrosis virus (PBNV) polyclonal antibodies

control (Table 4 and graphically represented in Fig. 2). The concentration of antigen used was 1:10000 and ALP 1:2000 concentration was used.

GBNV was detected in the inoculated samples under glasshouse condition (Table 4). The systemic symptoms of the inoculated cowpea (Cv.152) recorded highest virus concentration of about 1.60 OD value. Highest OD values were recorded in plants showing symptoms by leaves, buds with initial chlorosis symptoms, tender leaves with black spots. All tested plant parts showed considerably high OD values when compared to healthy and buffer control. The results indicated that high OD values were obtained where the virus concentration was more. The OD values of buffer control and healthy control were very low indicating no reaction to the host proteins.

4.3.2 Diagnosis by RT-PCR

A DNA fragment of ~830bp corresponding GBNV-CP gene was amplified from tomato leaf samples by RT-PCR using degenerate primers. The RT-PCR successfully amplified the CP-gene from tomato leaf samples collected from field. The infected leaf tissue provided a suitable template to use in RT-PCR and a major band of expected size (Lane 3-6) (~830bp) as compared to healthy samples (Lane-1 and 2) (Plate 2a).

4.3.3 Cloning and sequencing

Cloning

The coat protein gene was amplified by PCR to generate large quantities of DNA. The gel eluted coat protein gene fragment was ligated to vector pTZ57R/T of size ~830 bp with T- over hang. Transformation of *E. coli* strain DH5α with ligation mixture resulted in several colonies carrying the recombinant DNA on antibiotic (ampicillin) containing medium, which included recombinant as well as self ligated vector. White colonies obtained on the IPGT and X-gal plates were used for screening and recombinant clones. White colonies were produced due to the insertion of gene in multiple cloning sites, which resulted in disruption of *lacZ* expression. In the absence of insert, the *lacZ* gene is switched on by adding the inducer isopropyl β-D-thiogalactopyranoside (IPGT). Its presence, causes the enzyme β-galactosidase to be produced. The functional enzyme is able to hydralyse a colourless substance 5- bromo 4-chloro 3-indolyl- β-galactopyranoside (X-gal) to a blue insoluble material. Thus, bacteria carrying recombinant plasmids are unable to synthesize functional β-galactosidase enzyme producing white coloured colonies. Hence, bacterial colonies carrying the recombinant DNA molecules are colourless and can be easily identified from the non-recombinant blue colonies.

The clones carrying the coat protein gene were further analyzed and confirmed by colony PCR which produced about ~830 bp product as resolved and analyzed by gel electrophoresis.

Sequencing of the coat protein gene

The clones carrying the coat protein gene of GBNV was sequenced using M13 universal forward and reverse primers. The results revealed that the cloned DNA fragment of ~831 bp long nucleotide sequencing in Dharwad isolates. The data revealed that the coat protein gene had codons for 276 amino acids. The results of the BLAST (www.ncbi.nih.gov/BLAST) search performed to identify sequence homology, clearly demonstrated that the coat protein sequence matched with the reported GBNV coat protein gene sequences from different geographical locations of India. The nucleotide and translated to aminoacid sequences of coat protein (CP) gene of tomato Tospovirus isolate are presented in Tables 5a and b.

4.3.4 Diagnosis of disease by Electron Microscope (EM)

The partial purified preparations of virus were fixed in 1.5 per cent gluteraldehyde. Similar procedure was used for crude infected plant extract. The fixed preparations were stained with 1 per cent uranyl acetate and particles were observed under JEOL 100 X

Transmission electron microscope at magnification 24000x. Under this spherical particle of about 80-100 nm size were observed (Plate 2b).

4.4 TRANSMISSION

4.4.1 Standardisation of extraction buffer for transmission of GBNV Tospovirus

To know the effect of concentration of potassium phosphate buffer on the infectivity of GBNV, infected tomato leaves were taken and crude extracts were prepared using the buffers of different components. This crude extracts were used for sap inoculation to the 10 days old cowpea seedlings. The plants were kept in controlled condition for 4-5 days for symptom expression.

From the Table 6, it is clearly understood that the plants inoculated with 0.05 M potassium phosphate buffer (pH7) containing 0.02M mercaptoethanol showed 100 per cent transmission. Where as the same buffer containing different additives *viz*., 0.01 M thioglycerol and 0.01 Na_2SO_3 showed 56.25 per cent and 40.62 per cent transmission. The 0.1M potassium phosphate buffer containing 0.01 M Na_2SO_3 as additive also results in 26.92 per cent transmission.

4.4.2 Mechanical transmission

The crude leaf extracts prepared from GBNV infected young leaves and inoculated onto the local lesions hosts viz., *Vigna unguiculata*. cv. C-152, *Chenopodium amaranticolor, Chenopodium quinoa, Datura stramonium and Nicotiana tabacum* by mechanical inoculation the results obtained were noted.

The inoculated virus produced local lesions within 4-5 days of inoculation on cowpea, 3-4 days on Petunia plants. The percentage of transmission varied in different hosts. The transmission percentage ranged 90-100 per cent on *Chenopodium quinoa* but 100 per cent transmission was observed on cowpea, Cv.C-152, *C. amaranticolor* on *Datura stramonium* and on *Petunia hybrida* (Table 7).

4.4.3 Thrips transmission

Larvae and two days old adult *Thrips palmi* and *Scirtothrips dorsalis* were used to test the vector transmission of GBNV on Tomato. Larvae and adult of *T.palmi* were able to transmit the GBNV on tomato (Table 8) The per cent transmission by adult was 12.5 per cent, whereas by larvae it was 20 per cent. And symptoms were noticed on tomato after 20 days of transmission. However both larvae and adults of *S. dorsalis* failed to transmit and produce the symptoms on tomato.

4.5 HOST RANGE

4.5.1 Host range of GBNV tomato isolate

Twenty eight host plant species belong to 21 genera in 6 families (*Amaranthaceae, Asteraceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Solanaceae*) were tested in order to find out the host range of GBNV of tomato of Dharwad isolate by mechanical sap inoculation as described under the “Material and Methods”. The symptoms exhibited are presented in Table 9.

GBNV-Tomato (Dharwad) could infect members of all the families tested. However, there were small differences with respect to production of symptoms on various host species and the number of days taken for producing various types of symptoms.

Among 28 hosts, sunflower and chrysanthemum were not infected and 10 host plants showed systemically, whereas other hosts produced local symptoms.

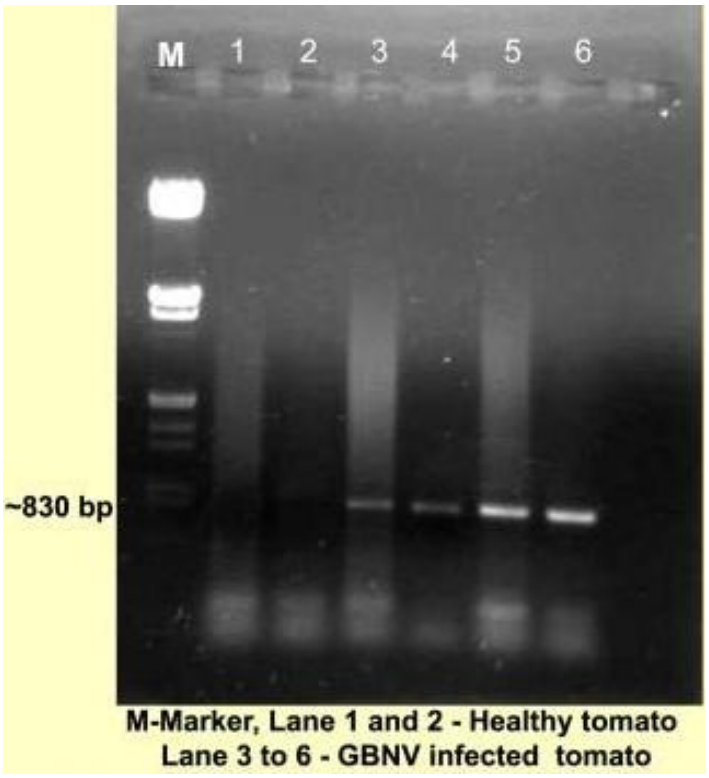


Plate 2. Agarose gel electrophoresis analysis of RT-PCR amplified product of C-DNA of GBNV

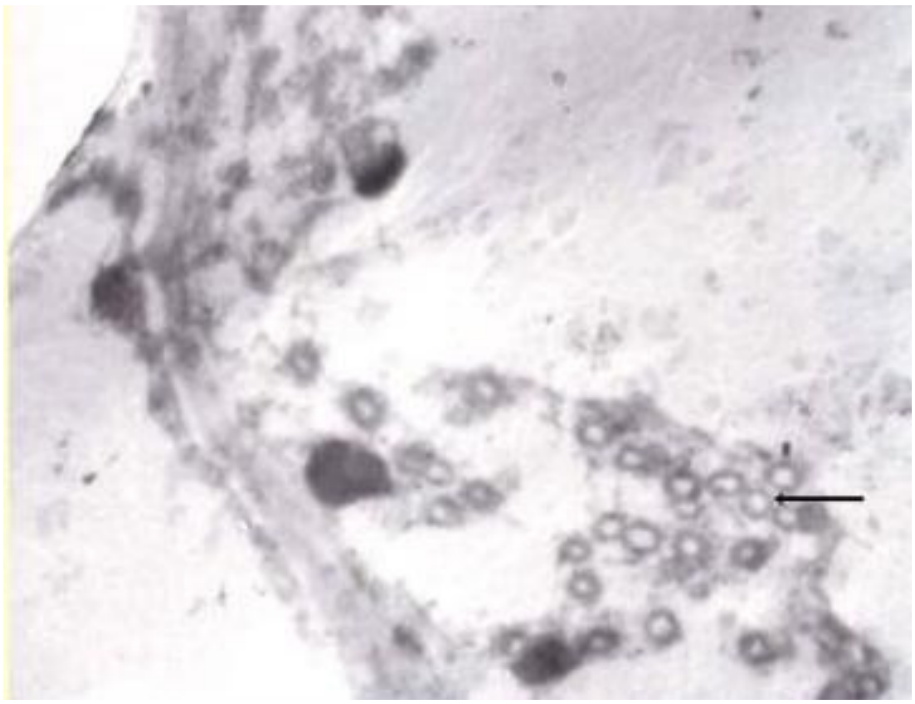


Plate 2b. Electron micrograph of spherical particles of GBNV at 24000 x

Table 5a. Deduced Coat protein gene sequence of GBNV tomato Dharwad isolate

0	ATG TCT ACC GTT AAG CAG CTC ACC GAG AAA AAA ATC AAA GAA CTT	15
	M S T V K Q L T E K K I K E L	
16	TTG GCT GGT GGC TCT GCA GAT GTT GAA ATT GAA ACA GAA GAT TCC	30
	L A G G S A D V E I T E D S T	
31	ACT CCA GGA TTT AGC TTT AAA GCT TTC TAT GAC ACT AAC AAA AAT	45
	P G F S F K A F Y D T N K N L	
46	CTT GAA ATA ACT TTC ACA AAC TGT TTG AAT ATT TTG AAG TGC AGG	60
	E I T F T N C L N I L K C R K	
61	AAG CAG ATC TTT GCT GCT TGT AAA AGT GGT AAG TAT GTT TTT TGT	75
	Q I F A A C K S J K Y V F C G	
76	GGT AAA ACT ATT GTT GCT ACA AAT ACT GAT GTA GGG CCA GAT GAC	90
	K T I V A T N T D V G P D D W	
91	TGG ACC TTC AAA AGG ACA GAA GCT TTC ATC AGA ACC AAA ATG GCT	105
	T F K R T E A F I R T K M A S	
106	AGT ATG GTT GAG AAG AGC AAG AAT GAT GCT GCT AAG CAG GAG ATG	120
	M V E K S K N D A A K Q E M Y	
121	TAC AAT AAA ATA ATG GAA TTA CCA TTA GTG GCA GCT TAT GGA TTA	135
	N K I M E L P L V A A Y G L N	
136	AAT GTT CCA GCA TCT TTC GAT ACA TGT GCT TTA AGG ATG ATG CTT	150
	V P A S F D P C A L R M M L C	
151	TGC ATT GGA GGT CCT CTG CCT CTC TTG TCT AGC ATG ACA GGT CTG	165
	I G G P L P L L S S M T G L A	
166	GCA CCA ATT ATA TTC CCT CTG GCT TAT TAT CAA AAT GTG AAG TCA	180
	P I I F P L A Y Y Q N V K S E	
181	GAG AAA TTA GGA ATT AAG AAC TTT TCT TAT TAT GAA CAG GTT TGC	195
	K L G I K M F S Y Y E K V C K	
196	AAA GTA GCC AAA GTG CTT CAT GCT TCA CAG ATT GAG TTC AAA AAT	210
	V A K V L H A S Q I E F K N E	
211	GAA CTA GAG GAA ATG TTT AAA TCA GCT GTA AAG CTA TTG AGT GAG	225
	L E E M F K S A A V K L L S E	
226	AGC AAC CCT GGA ACA GCC AGC TCT ATC TCA CTC AAG AAA TAT GAT	240
	S N P G T A S S I S L K K Y D	
241	GAA CAA GTC AAA TAC ATG GAC AAA GCT TTC AGT GCC AGT CTC TCA	255
	E Q V K Y M D K A F S A S L S	
256	ATG GAT GAT TAT GGT GAA CAT TCT AAG AAG AAG AGT TCA AAG GCT	270
	M B D Y G E H S K K K S S K A	
271	GGT CCT TCG TTG GAA GTG TAA	276
	G P S L E V <	

Table 5b. Comparison of GBNV tomato Dharwad isolate with other GBNV isolates in India

Sl. No.	Virus accession number	Hit results	Query coverage	E-value (%)	Sequence homology (%)	Authors	Submission
1	AY184354.1	Peanut bud necrosis virus nucleocapsid protein mRNA, complete cds	100	0.0	98	Savithri, H.S.	Submitted (20-19-2002), biochemistry IISc, Malleshwaram, Bangalore
2	U27809.1	Peanut bud necrosis virus segment S non-structural protein in nucleocapsid protein genes, complete cds	100	0.0	97	Mitchel S.C.	Sumitted (26-5-1995) Sharon Mitchell, PGRC, USDA/ARS, Univ. Georgia, Experiment Station, Griffiri
3	AY727923.1	Groundnut bud necrosis virus nucleocapsid protein (NP) gene complete cds	100	0.0	97	Raja, P.	Submitted (18-14-2004) plant pathology, IARI, Pusa Campus, New Delhi, Delhi-110012, India
4	AF515820.1	Peanut bud necrosis virus isolate potato Madhya Pradesh nucleocapsid protein (N) mRNA, complete cds	100	0.0	97	Jain, R.K.	Sumitted (27-50-2002) division of plant pathology, IARI, Pusa, New Dlehi, Delhi-110012, India
5	AY510134.1	Peanut bud necrosis virus nucleocapsid, protein gene, complete cds	100	0.0	98	Ravi, K.S.	Submitted (22-12-2003), molecular virology mahyco life sciences Research centre, Jalna-Aurangabad, Road, Dawalwadi-431203, India
6	EU373784	Peanut bud necrosis virus isolation-source tomato nucleocapsid protein mRNA, complete cds	100	0.0	98	Ravi, K.S.,	Submitted (06-1-2008) molecular virology mahyco life sciences Research centre, Jalna-Aurangabad, Road, Dawalwadi-431203, India

Table 6. Effect of different concentration of inoculation buffers on the infection of GBNV on cowpea

Sl. No.	Buffers used	Additives used	Abbressive used	No of cowpea plants inoculated	No. of cowpea plants infected	Per cent transmission
1	0.05M KPO_4 pH.7.0	0.02mM Mercaptoethanol	Carborendum	32	32	100
2	0.05M KPO_4 pH.7.0	0.1M Thioglycerol	Carborendum	32	18	56.25
3	0.01M KPO_4 pH 7.0	0.01M Na_2SO_3	Carborendum	32	13	40.62
4	0.05M KPO_4 pH, 7.0	0.01M Na_2SO_3	Carborendum	26	7	26.9

Table 7. Sap transmission of GBNV of tomato on five local lesion host species

Sl. No.	Host species inoculated	Number of plants inoculated	Number of days taken for expression symptoms	Total number of plants infected	Per cent transmission of GBNV
1	*Vigna unguiculata* (L) cv. C-152	22	4-5	22	100
2	*Chenopodium amaranticolor* L.	30	8-10	29	96.6
3	*Chenopodium quinoa* .L	32	7-8	31	96.87
4	*Datura stramonium*	30	6-8	30	100
5	*Petunia hybrida* (Coral satin)	30	3-4	30	100

Table 8. Vectors transmission of GBNV on tomato

Thrips species tested	Stage of thrips	No. of thrips vector used in transmission	No. of plant inoculated	No. of days required for expression of symptoms	No. of plants infected	Per cent transmission
Thrips palmi	Larvae	10	10	22-25	2	20
	Adult	10	8	22-25	1	12.5
Scirtothrips dorsalis	Larvae	20	10	--	0	0
	Adults	15	5	--	0	0

The virus induced chlorotic local lesions on *Chenopodium album, Citrullus lanatus, Cucumis melo, Luffa acutangula, Cucumis sativus Arachis hypogaea, Vigna unguiculata, Capsicum annuum, Solanum lycopersicum, Nicotiana benthamiana* and neorotic local lesions on *Chenopodium amaranticolor, Amaranthus retroflexus., Amaranthus viridis, Petunia hybrida, Vigna mungo*.

The virus induced systemic lesions on *Amaranthus retroflexus*, necrosis and ring spots on *Arachis hypogea.* Yellowing, mottling and mosaic symptoms on *Vigna radiata.* Mosaic, necrosis, chlorotic ring spots and necrotic local lesions on *Capsicum annum* and necrotic local lesions on *Capsicum* spp. Mottling, chlorotic spots, on *Datura stramonium* lesions, mottling, mosaic symptoms on *N. benthianum*. The yellowing and chlorotic spots on French bean. The 2 Systemic Latent hosts are *N.glutinosa* and *N. tabaccum*. The non host includes *Helianthus annuus* and *Chrysanthemum indicum,* the ring spots produced on *Emelia sonchifolia* (Plate 3 to 7).

4.6 MANAGEMENT OF BUD BLIGHT

4.6.1 Screening of varieties/hybrids against bud blight

Plants showing characteristic necrotic symptoms stems and leaves were considered for disease scoring. The level of infection among the different varieties varied from 10-100 per cent. The reactions of tomato varieties/hybrids to GBNV infection during summer 2008 revealed that, out of 22 varieties / hybrids screened under field condition at Saidapur farm, UAS, Dharwad, none of them were resistant to GBNV. The variety marikrit showed moderately resistant reaction (10%), NS-2535 found to be mdoeratley susceptible (15%) and PKM-1, Vybhav were two found to be susceptible. The disease reaction of screened varieties are presented in Tables 10 and 11.

Among 22 varieties/hybrids, 7 were namely tomato hybrid-530, PKM-1, NS-658, NS-719, NS-585, Malini, Utsav expressed symptoms very early (10-20) and remaining 15 varieties/hybrids expressed symptoms early (21-40).

4.6.2 Antiviral principle studies

4.6.2.1 Effect of leaf extract on incidence and enzymatic activity against GBNV

Isozymes are the molecular form with similar or identical substrate specificity occurring within the same organism. The difference in the isozyme activity can be used as a laboratory tool for the biochemical characterization of different plant extracts against GBNV, in this study cowpea plants were used to estimate the phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase isozyme activities against GBNV using 10 per cent coconut and sorghum leaf extracts.

a. Coconut leaf extract spray (10%)

Spraying of coconut leaf extract (10%) inhibited the GBNV infectivity and least lesion production (3.0/leaf) two days after virus inoculation. Average number of lesions on the different treatments ranged from 3 to 42.5, maximum lesions were noticed on T_4 (42.5) and minimum number of lesions were recorded in T_2 (3.0).

The results revealed that, maximum enzymatic activity in T_2 (4.25) followed by T_1 (3.35) and minimum enzymatic activity was recorded in T_5 (1.97). Among the enzymes maximum activity was recorded by PAL in T_2 (9.53) and minimum activity was recorded by PPO in T_5 (0.37).

The interaction effect revealed that the maximum enzymatic activity was recorded by PAL in T_2 (9.53) followed by PAL in T_1 (7.59) and least enzymatic activity recorded by PPO in T_5 (0.37) (Table 12).

Table 9. Host range of GBNV of tomato

Sl. No.	Name of the Host Plants	% transmission	No of days required to express symptoms	Local	Systemic	Virus recovery on cowpea cv. C-152		Remarks
						Local	Systemic	
1.	*Amaranthaceae*							
	a. *Amaranthus viridis*	83.3	8-10	NLL	--	--	--	LLH
	b. *Amaranthus retroflexus*	75	12-13	CLL	CS	--	--	LSH
	c. *Amaranthus spp.*	50	10-12	NLL	--	--	--	LLH
	d. *Alternanthera sessiles*	100	7-8	NS.Ld	--	--	--	LLH
2.	*Asteraceae*							
	a. *Chrysanthemum indicum*	--	--	--	--	--	--	NH
	b. *Emilia sonchifolia*	85	16-18	NRS	--	--	--	LLH
	c. *Helianthus annuus*	--	--	--	--	--	--	NH
3.	*Chenopodiaceae*							
	a. *Chenopodium album*	80	8-10	CLL	--	--	--	LLH
	b. *Chenopodium amaranticolor*	100	7-9	NLL	--	--	--	LLH
	c. *Chenopodium quinoa*	80	10-11	NLL	--	--	--	LLH
4.	*Cucurbitaceae*							
	a) *Citrullus lanatus* cv. Arka manik	20	15-18	CLL	--	+	-	LLH
	b) *Cucumis melo*	50	20-22	CLL	--	+	-	LLH
	c) *Luffa acutangula.*	70	20-22	CLL		+	-	LLH
	d) *Cucumis sativus*	70	20-22	CLL	--	+	-	LLH

Table 9. Contd……..

5	*Fabaceae*							
	a. Arachis hypogaea cv. JL-24	10	14-16	CLL	NS,RS	--	+	LSH
	b. Pisum sativum.							
	c. Vigna radiata	--	--	--	--	--	-	NH
	d. Vigna mungo	100	10-12	M,M., & CLL	Y	+	+	LSH
	e. Vigna unguiculata	70	10-12	NRS	--	--	-	LLH
	cv. C -152	100	4-5	CLL	Ld, LSH	+	+	LLH
	f. Phaseolus vulgaris	100	10-12	CS	-	-	-	LSH
6	*Solanaceae*							
	a. Capsicum annuum cv. Byadagi kaddi	100	15-20	CR,NLL	M, NS	-	-	LSH
	b. *Capsicum spp.* (Paprika chilli)	100	15-20	CLL, NLL,	Br, Ns	-	-	LSH
	c. Datura stramonium	100	8-10	CLL,	Mo, CS	+	+	SLH
	d. Solanum lycopersicum cv. Megha	80	20-22	NLL, CLL	--	+	-	LLH
	e. Nicotiona benthamiana	90	20-22	CLL, Mo, CS	Ld	-	-	LSH
	f. Nicotiana glutinosa	100	20-22		Ld	-	-	SLH
	g. Nicotiana tabacum	100	18-20	Mo, M	-	-	-	SLH
	h. Petunia hybrida (cv. Coral satin)	100	4-5	CLL, Mo NLL	-	-	-	LLH

NLL-Necrotic local lesion, M-Mosaic, Mo-Mottling, Ns-Necrosis, Y-Yellowing, CS-Chlorotic spots, CLL-Chlorotic local lesions, Ld-Leaf distortion, NRS-Necrotic ringspots, LLH-Local lesions host, LSH-Local systemic host, SLH-Systemic latent host, RS-Ringspots, NH-Non-host, CR-Chlorotic rings

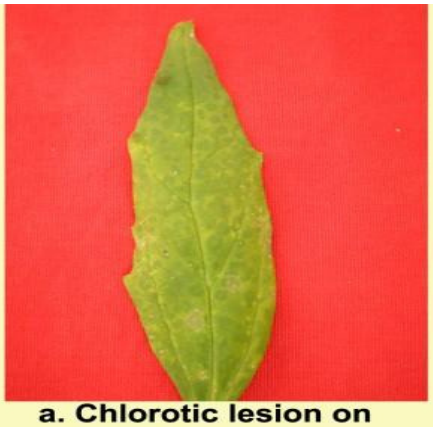
a. Chlorotic ring spot on groundnut leaf

b. Chlorotic lesions on cowpea

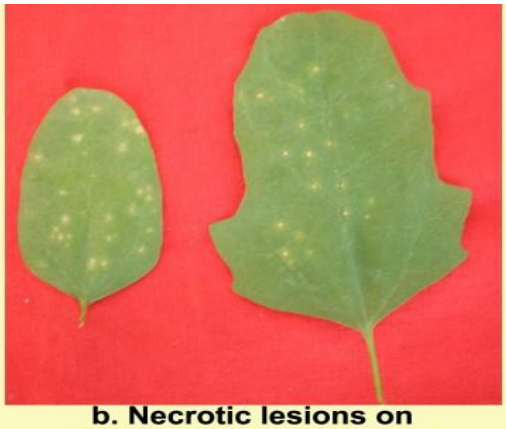
c. Systemic symptoms on cowpea

d. Chlorotic and necrotic rings spots on chilli

Plate 3. Symptoms of GBNV on groundnut, cowpea and chilli leaves



a. Chlorotic lesion on *Chenopodium album*



b. Necrotic lesions on *Chenopodium amaranticolor*

c. Necrotic lesions on *Chenopodium quinoa*

d. Chlorotic lesions on *Datura stramonium*

Plate 4. Symptoms of GBNV on *Chenopodium* spp. And datura

a. Chlorotic local lesions on *Cucumis sativus*

b. Chlorotic local lesions on *Luffa acutangula*

c. Chlorotic local lesions on *Nicotiana benthamiana*

Plate 5. Symptoms of GBNV on *cucumis sativus, Luffa acutangula, Nicotiana benthamiana*

a. Systemic symptoms on *Nicotiana glutinosa*

b. Lesions on *Nicotiana tabacum*

C. Necrotic lesions on *Petunia hybrida*

Plate 6. symptoms of GBNV on tobscco dpecies and *Petunia hybrida*

a. Necrotic ring spots on *Alternanthera sessiles*

b. Necrotic lesions on *Emelia sonchifolia*

c. Chlorotic lesions *Phaseolus vulgaris*

d. Necrotic local lesions *Amaranthus viridis*

Plate 7. Symptoms of GBNV on Alternanthera sessiles Emelia sonchifolia, Phaseolis vulgaris and Amaranthus viridis

Table 10. Response of tomato varieties/hybrids against bud blight disease during summer - 2008

Sl. No.	Variety / hybrid	Symptom expression	Per cent disease incidence	Disease reaction	Thrips population/ plant
1	Nandi	E	26.9	HS	1.00
2	NS-2535	E	15.0	MS	0.66
3	NS-816	E	77.7	HS	1.66
4	NS-2532	E	28.2	HS	1.00
5	Megha	E	72.2	HS	1.00
6	NS-564	E	85.7	HS	1.00
7	Tomato hybrid – 530	VE	64.2	HS	1.00
8	PKM-1	VE	22.8	S	2.00
9	Vybhav	E	20.0	S	2.33
10	NS-53	E	66.6	HS	2.00
11	Sankranti	E	70.0	HS	1.66
12	NS-658	VE	68.4	HS	1.66
13	NS-719 (519)	VE	58.5	HS	0.66
14	Sonam	E	40.0	HS	1.66
15	NS-585	VE	42.0	HS	1.33
16	NS-812	E	100	HS	3.00
17	Malini	VE	85.7	HS	1.00
18	Utsav	VE	30.2	HS	2.00
19	NS-563	E	100	HS	3.00
20	Arka Vikas	E	12.0	HS	2.33
21	VC-2048	E	85.7	HS	1.33
22	Marikrit	E	10.0	MR	0.66

Table 11. Grouping of genotypes into different categories against bud blight on tomato during summer 2008

Sl. No.	Categories	Genotypes
1	Resistant	--
2	Moderately resistant	Marikrit
3	Moderately susceptible	NS-2535
4	Susceptible	PKM-1, Vybhav
5	Highly susceptible	Nandi, NS-816, NS-2532, Megha, Ns-564, Tomato hybrid -530, NS -53, Sankranti, NS-658, Ns-719 (519), Sonam, NS-585, NS, 812, Malini, Utsav, Ns-563, Arka Vikas,, VC-2048.

Table 12. Effect of coconut leaf extract (10%) and sorghum leaf extract (10%) on GBNV

Treatments	Coconut leaf extract (10%)					Sorghum leaf extract (10%)				
	Average no of lesions /leaf.	PAL*	PO*	PPO*	Mean	Average no of lesions /leaf.	PAL*	PO*	PPO*	Mean
Spray leaf extract- after 24 hrs inoculation - observed for symptoms development – T_1	11.25	7.59	1.57	0.88	3.35	12.5	7.98	1.62	0.78	3.46
First inoculation -after 48 hrs spray leaf extract-observed for symptoms development – T_2	3.0	9.53	1.84	1.37	4.25	3.5	10.14	1.98	1.20	4.44
First inoculation-after 48 hrs spray-leaf extract- after 24 hrs inoculation-observed for symptoms development – T_3	38.5	7.42	1.48	0.82	3.24	28.75	7.46	1.29	0.58	3.11
Inoculation- No spray - observed for symptoms development – T_4	42.50	4.75	1.73	0.56	2.34	42.75	4.79	1.72	0.58	2.36
Control - No spray No inoculation -observed for symptoms development – T_5	-	4.27	1.10	0.37	1.97	-	4.24	1.22	0.39	1.95
Mean		6.71	1.54	0.80	3.03		6.92	1.57	0.71	3.07
	S.Em±		CD@1%			S.Em±		CD @ 1%		
Treatment (T)	0.12		0.45			0.11		0.41		
Enzymes (E)	0.09		0.34			0.09		0.34		
TxE	0.20		0.79			0.19		0.72		

* mg protein/min/g of sample, PAL – Phenyl alanine ammonia lyase, PO – Peroxidase, PPO – Polyphenol oxidase

b. Sorghum leaf extract (10%)

Spraying of sorghum leaf extract (10%) inhibited the GBNV infectivity and least lesion production (3.5/leaf) two days after virus inoculation.

Average number of lesions recorded was ranging from 3.5 to 42.75, maximum number of lesions produced on T_4 (42.75) minimum number of lesions was recorded in T_2 (3.5) (Table 12).

The results revealed that maximum enzymatic activity in T_2 (4.44) followed by T_1 (3.46) and least enzymatic activity was recorded in T_5 (1.95). Among enzymes PAL was recorded highest enzymatic activity (10.14), least enzymatic activity was recorded by polyphenol oxidase (0.39). The interaction effect revealed that the maximum enzymatic activity recorded by PAL in T_2 (10.14) followed by PAL in T_1 (7.98) and least enzymatic activity recorded by PPO in T_5 (1.95).

It is clear from this study that AVP from sorghum and coconut leaf extracts were effective in inhibition of virus infection only when applied 48 hrs after inoculation (Table 12).

4.6.3 Management of bud blight in the field

4.6.3.1 Effect of different treatments on the incidence of GBNV in tomato

An experiment was conducted to know the effect of different management, practices to control the disease in the main field. The results revealed that at 20, 40, 60 and 80 DAT the average per cent disease incidence was significantly higher in untreated control T_9 (48.29%) followed by T_{10} (40.13%) where package of practices (POP) was used. Least per cent disease incidence was recorded on T_6 (16.54%) followed by T_4 (23.09). The disease incidence was more at 80 DAT in untreated Control T_9 (92.22%) followed by T_{10} (71.64%) in POP used. Least incidence was observed at 20 DAT in T_4 (1.13%) which is on par with the T_1 (1.80%) and T_6 (1.55%). Interaction between treatments and number of days after transplanting differ significantly. The highest incidence was observed in untreated control of T_9 at 80 DAT (92.22%) followed by POP of T_{10} at 80 DAT (71.64%) and least incidence was observed in T_4 (1.13%) at 20 DAT which is on par with the T_6 (1.55%) and T_1 (1.80%) at 20 DAT (Table 13 and graphically represented in Fig. 3).

4.6.3.2 Effect of different treatments on the thrips population in tomato during summer 2008

In this experiment effect of different management practices in the reduction of thrips population was studied. The number of thrips per leaf was recorded (Table 14 and Fig. 4).

The results revealed that thrips population per leaf was more in untreated control T_9 (1.35) followed by T_{10} (1.11), T_8 (0.91) and T_6 (0.55) maximum thrips population was recorded at 60 DAT (1.72) followed by 40 DAT (1.24) per leaf and least population was observed at 80 DAT.

Table 13. Effect of different management practices on the incidence of bud blight in tomato under field condition during summer 2008

Sl. No.	Treatments	Dosage (a.i./ha)	Per cent disease incidence				Mean
			20 DAT	40 DAT	60 DAT	80 DAT	
1	T_1 - Seed treatments with imidachloprid 70 WS (0.005%)	Imidachloprid - 5 g/kg seeds	1.80 (6.21)	26.03 (30.41)	47.42 (43.46)	69.89 (56.73)	36.28 (34.20)
2	T_2 - Border crops with maize sown before transplanting (cv. South African tall)		6.53 (14.40)	30.70 33.56	42.70 (40.75)	58.83 (49.73)	34.54 (34.61)
3	T_3 - T_1 + spray of sorghum and coconut leaf extract (10%) at 15 days interval after transplanting alternatively.	Imidachloprid - 5 g/kg seeds, 20 l/spray	3.20 (9.70)	21.90 (27.85)	32.09 (34.43)	51.42 (45.79)	27.09 (29.44)
4	T_4 - T_2 + T_3	Imidachloprid - 5 g/kg seeds, 20 l/spray	1.13 (4.95)	17.10 (24.17)	30.86 (33.70)	43.25 (41.09)	23.09 (25.97)
5	T_5 - T_1 + Thiomethoxom 25 WG (0.05%)-4 spray at 15 days interval after transplanting	Imidachloprid - 5 g/kg seeds - 20 l/spray Thiomethoxam -0.5 kg/ha/spray	5.33 (12.93)	19.83 26.35	32.12 (34.45)	43.99 (41.51)	25.32 (28.81)
6	T_6 - T_1 + Ist spray of Intrepid (0.5%) + 2nd spray of Acetamiprid 20 SP (0.02%) + 3rd spray of Thiomethaxom 25 WG (0.05%) at 20 days interval after transplanting	Intrepid 500 ml/ha/spray, Acetamiprid - 0.75 kg/ha/spray, Thiomethoxam - 0.5 kg/ha/spray	1.55 (6.36)	13.09 (21.13)	20.08 (26.50)	31.45 (34.09)	16.54 (22.02)
7	T_7 - 8% alcohol-4 spray at 20 days interval after transplanting	Alcohol – 4 l/spray	4.35 (11.75)	24.16 (29.39)	35.81 (36.74)	43.05 (40.98)	26.84 (29.71)
8	T_8 - Imidachloprid 17.8 SL (0.005%) 4 sprays at 15 days interval after transplanting	Imidachlorpid -0.07 l/spray	4.98 (12.81)	22.89 (28.54)	33.08 (35.08)	40.63 (39.58)	25.39 (29.00)
9	T_9 - Control (No spray)	-	8.67 (17.03)	33.71 (35.43)	58.56 (49.92)	92.22 (74.04)	48.29 (44.10)
10	T_{10} - Package of practice	-	8.19 (16.55)	32.28 (34.60)	48.40 (44.06)	71.64 (57.82)	40.13 (38.26)
	Mean		4.57	24.17	38.11	54.58	30.35
Treatment (T)			S.Em±			CD at 5%	
DAT			0.72			2.03	
T x DAT			0.45			1.27	
Treatment (T)			1.44			4.05	

Values in parenthesis are transformed values, DAT – Days after transplanting

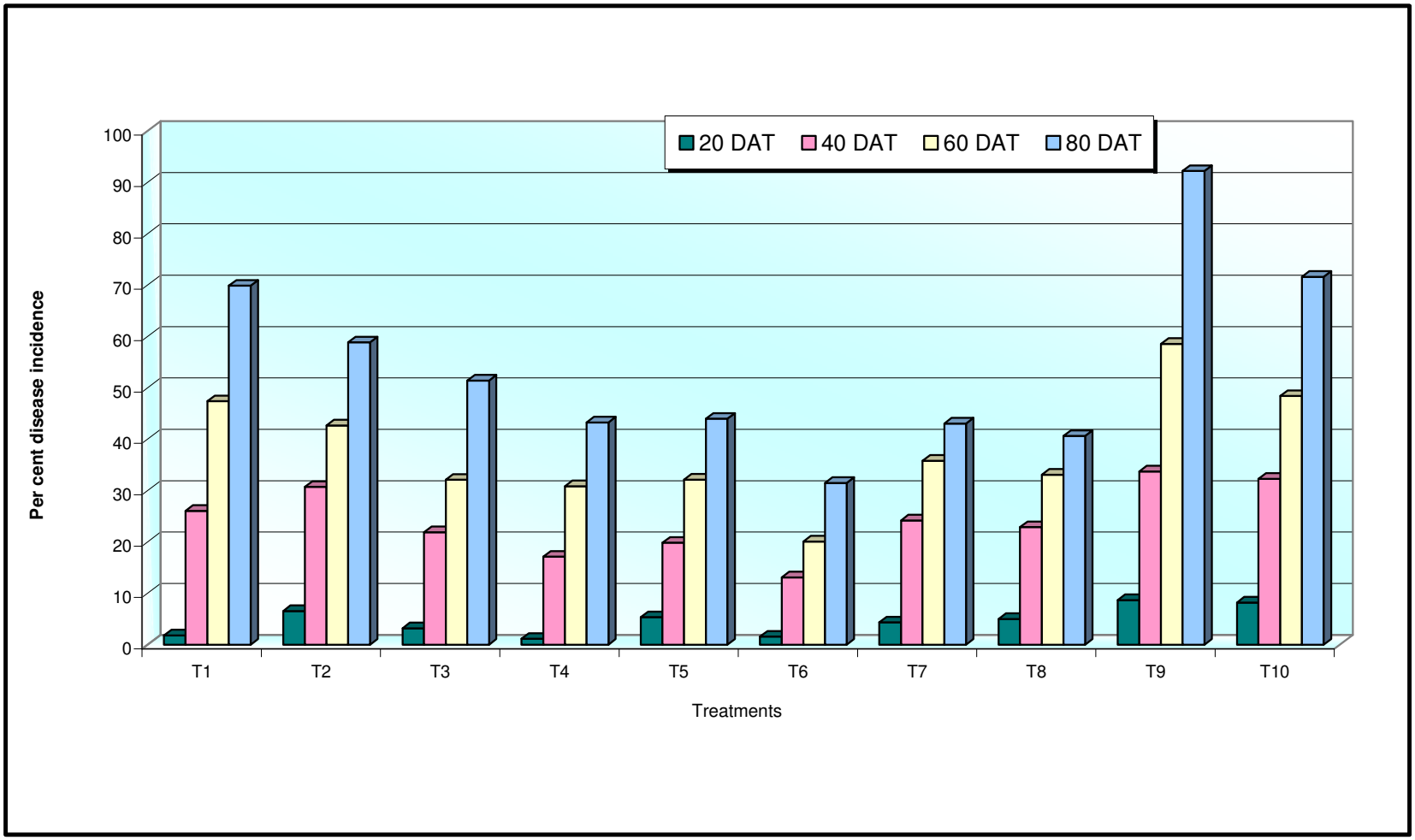


Fig. 3. Effect of different management practices on the incidence of bud blight in tomato under field condition during summer-2008

Table 14. Effect of different management practices on the thrips population on tomato under field condition during summer 2008

Sl. No.	Treatments	Dosage (a.i./ha)	Thrips population/leaf				Mean
			20 DAT	40 DAT	60 DAT	80 DAT	
1	T_1 - Seed treatments with imidachloprid 70 WS (0.005%)	Imidachloprid - 5 g/kg seeds	0.61	1.10	1.50	0.90	1.02
2	T_2 - Border crops with maize sown before transplanting (cv. South African tall)		0.37	1.42	1.93	0.48	1.05
3	T_3 - T_1 + spray of sorghum and coconut leaf extract (10%) at 15 days interval after transplanting alternatively.	Imidachloprid - 5 g/kg seeds, 20 l/spray	0.56	0.2	2.16	0.66	1.00
4	T_4 - T_2 + T_3	Imidachloprid - 5 g/kg seeds, 20 l/spray	0.00	1.33	2.05	0.53	0.97
5	T_5 - T_1 + Thiomethoxom 25 WG (0.05%)-4 spray at 15 days interval after transplanting	Imidachloprid - 5 g/kg seeds - 20 l/spray Thiomethoxam -0.5 kg/ha/spray	0.36	1.66	1.56	0.33	0.98
6	T_6 - T_1 + I^{st} spray of Intrepid (0.5%) + 2^{nd} spray of Acetamiprid 20 SP (0.02%) + 3^{rd} spray of Thiomethaxom 25 WG (0.05%) at 20 days interval after transplanting	Intrepid 500 ml/ha/spray, Acetamiprid - 0.75 kg/ha/spray, Thiomethoxam - 0.5 kg/ha/spray	0.33	0.65	0.66	0.56	0.55
7	T_7 - 8% alcohol-4 spray at 20 days interval after transplanting	Alcohol – 4 l/spray	0.32	0.82	2.10	0.68	0.98
8	T_8 - Imidachloprid 17.8 SL (0.005%) 4 sprays at 15 days interval after transplanting	Imidachlorpid -0.07 l/spray	0.40	1.57	1.33	0.35	0.91
9	T_9 - Control (No spray)	-	0.66	2.00	2.16	0.58	1.35
10	T_{10} - Package of practice	-	0.32	1.22	1.73	1.20	1.11
	Mean		0.39	1.24	1.72	0.62	0.99
Treatment (T)			S.Em±			CD at 5%	
DAT			0.04			0.12	
T x DAT			0.02			0.08	
Treatment (T)			0.08			0.25	

DAT – Days after transplanting

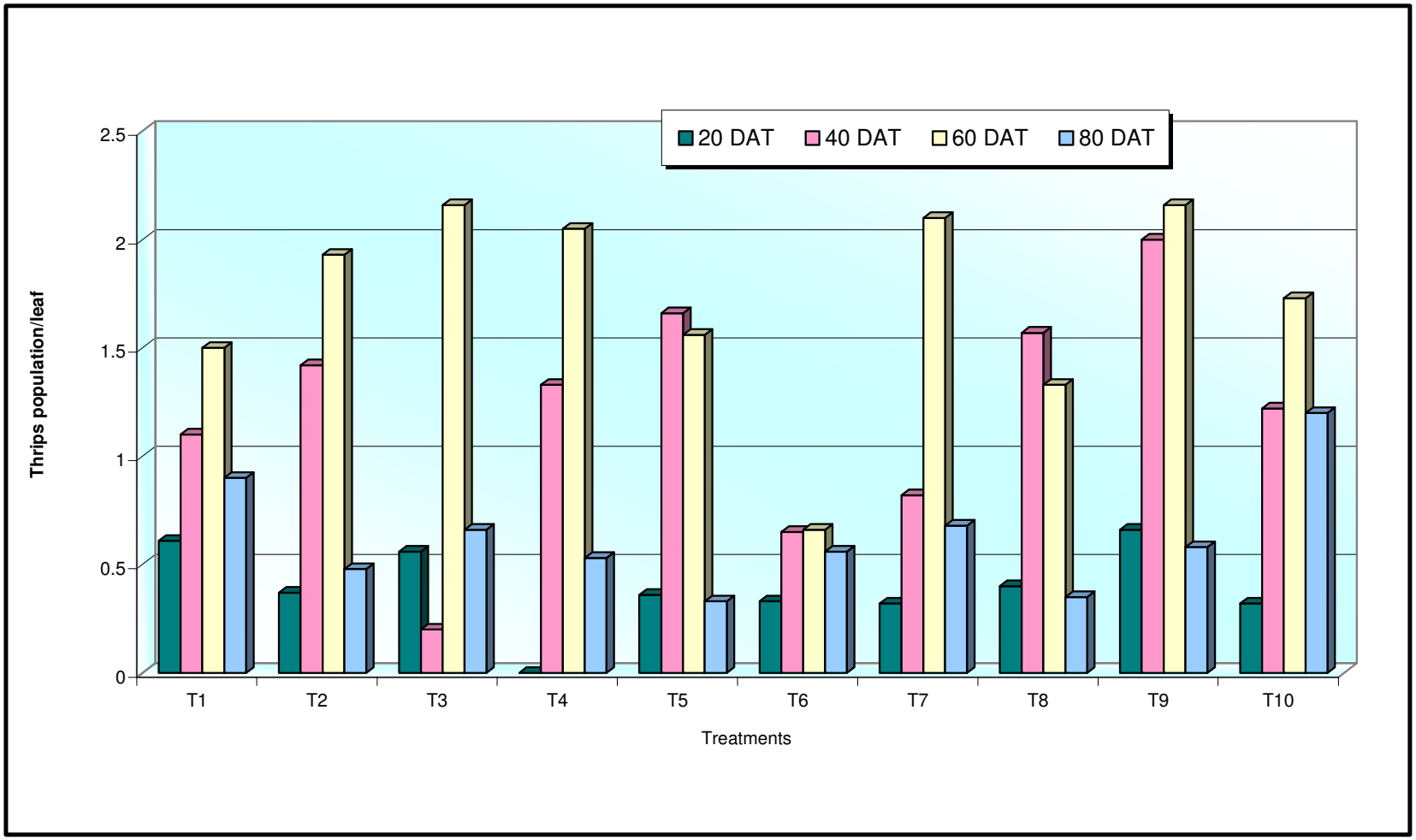


Fig. 4. Effect of different management practices on the thrips population on tomato under field condition during summer-2008

5. DISCUSSION

Bud blight disease on tomato caused by GBNV is one of the most important viral diseases of tomato in Karnataka and causes severe losses every year. The GBNV produces chlorotic, necrotic ring spots followed by occurrence of necrotic streaks on stem, leaves and petioles. Wilting and the death of the affected plants were common. Symptoms under natural and artificially inoculated conditions were identical. The similar type of symptoms were reported to the earlier reports of TSWV on tomato (Todd *et al.,* 1975; Prasad Rao *et al.,* 1980; Sastry, 1982). The above symptoms can differentiate this virus from mosaic, leaf curl, yellowing and leaf distortion caused by other viruses like cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), and tomato leaf curl virus.

Earlier TSWV was considered a monotypic virus in the Tospovirus group. Presently, there are many tospoviruses on several crops including tomato, and occurrence in vegetable crops is increasing year by year (Krishna Reddy *et al.,* 1997). The disease exists, since from several decades threatening the commercial cultivation of tomato particularly during summer due to intensive cultivation and use of hybrid tomatoes. The management strategies needed to be changed from time to time.

Considering the importance of bud blight disease, keeping all the above mentioned points and several studies were undertaken at Department of Plant Pathology, UAS, Dharwad. Survey for the incidence of GBNV on tomato in different tomato growing areas of North Karnataka, its symptomatology, detection of the pathogen by transmission, ELISA, PCR, cloning and sequencing and electron microscopy. Screening of available varieties and hybrids and management of bud blight disease.

The symptoms observed were chlorosis and necrosis on the new growth, reduction in plant growth and necrotic lesions on stem and petioles, infected stems become brittle and there will be production of chlorotic rings on the fruit. The disease can cause death of the plant or drastic reduction in marketable yield of the fruits (Cuppertino *et al.,* 1984; Diez *et al.,* 1993). The virus was recently identified as groundnut bud necrosis virus (GBNV) on the basis of nucleo capsid protein (N) gene characterization (Umamaheswaran *et al.,* 2003).

5.1 SURVEY IN DIFFERENT TOMATO GROWING AREAS FOR DISEASE INCIDENCE

The survey was conducted to assess the incidence of budblight in major tomato growing areas of Karnataka *viz*., Belgaum, Dharwad, Haveri and Kolar districts. The results revealed that the per cent incidence varied from location to location and variety to variety. However, the bud blight disease and the vector thrips were found in almost all the tomato fields surveyed. During summer season the incidence varied from 12.5-94.4 per cent. The higher incidence of GBNV during summer months could be attributed to higher thrips and their active dispersal population. Similar observations were recorded by Prasad Rao *et al.* (1980) and Singh and Tripati (1991).

The disease appeared in a severe form in early infected crops due to improper protection at nursery stages and also improper management practices in the main field. All the varieties or hybrids grown in the surveyed region were found to be susceptible to the virus. However, hybrids were more susceptible than the varieties. The varieties grown in Belgaum were Mahyco hybrid, Rashmi, Utsav, NS-2535 Ashoka seeds and Seminis hybrids. In Dharwad, Megha, PKM-1, Sungro, Mahyco hybrids, local and Rashmi varieties were grown. In Haveri district major varieties grown were Utsav, Mahyco hybrids, PKM-1 and Sungro hybrid seeds. In Kolar district major varieties were Abhinav, PKM-1, NS-618. Among the surveyed areas the incidence was higher in Kolar, the potential commercial tomato growing area, where mostly hybrids were grown followed by Belgaum, Haveri and Dharwad. In Kolar, in some of the experimental plots where no plant protection measures were adopted the disease incidence up to 94.4 per cent was noticed on Abhinav hybrid.

5.2 DIAGNOSIS OF DISEASE BY ELISA/PCR/CLONING AND SEQUENCING/EM

5.2.1 Enzyme Linked Immunosorbent Assay (ELISA)

Direct antigen-coated enzyme linked immunosorbent assay (DAC-ELISA) was employed by using PBNV antiserum (polyclonal) to detect the presence of GBNV in different hosts by mechanically inoculated plants under glasshouse condition. The GBNV pathogen was detected by DAC-ELISA in all the samples tested utilizing crude sap of the samples. However, the value of absorbance was varied with sample to sample, which may be due to variation in the concentration of the virus in the inoculated samples. This variation in concentration may be due to varietal difference of different hosts and also due to age of the crop.

Serological techniques such as ELISA, ISEM and DIBA have been used for the detection and differentiation of tospoviruses using both monoclonal and polyclonal antibodies (Hobbs *et al.,* 1987; Adam *et al.,* 1991).

5.2.2 RT-PCR

The infected tomato leaf tissue provided a suitable template to use in RT-PCR and a major band of expected size (~830 bp) was obtained. The serological and PCR based detection of GBNV virus suggested the association of an isolate of GBNV with bud blight disease of tomato. The result is in confirmation with earlier findings the samples collected from Kerala (Umamaheshwaran *et al.,* 2003), and from Pune and Rahuri (Maharastra), Coimbatore (Tamil Nadu) and Kanpur (UP) were also found associated with GBNV (Raja and Jain, 2006). From the present study and previous review it can be concluded that the GBNV infecting tomato is prevalent in different parts of India, including north Karnataka.

5.2.3 Cloning and sequencing

The identity of ~830 bp product was confirmed by cloning and sequencing. The CP gene of tomato Tospovirus and 831 nucleotides long and could potentiality code for a protein of 276 amino acids, which was 1 and 30 amino acids longer than that of corresponding genes of WBNV (Jain *et al*., 1998) and GYSV (Satyanarayana *et al*., 1998) respectively. Isolates in the Tospovirus genus with greater than 90 per cent nucleocapsid sequence homology are considered as strains of the same virus (Goldbach and Kuo, 1996; Moyer, 1999). Comparative sequence homology in the present study has shown that the tomato Tospovirus shared maximum sequence homology with GBNV at nucleotide (97%) as well as amino acid (99%) levels. Close sequence relationship between the CP gene of tomato Tospovirus and GBNV is in agreement with serological data. Since the CP gene sequence similarity of tomato Tospovirus with GBNV was above the threshold level of 90 per cent, it is proposed that the tomato Tospovirus should be regarded as a strain of GBNV, henceforth designated as GBNV-To Dharwad isolate.

This sequence studies may have direct bearing on the management of virus diseases either by cross protection or by coat protein mediated resistance as such the CP gene mediated resistance is highly sequence specific as observed by Lomonossoff (1995).

5.2.4 Electron microscopy

Detection of Tospovirus particles and their identification by electron microscopy can usually be achieved by examination of leaf dip preparation or of thin sections of infected cells (Milne, 1970). Fixation is found to be essential to avoid flattening and distortion of particles before staining (Francki and Halta, 1981). Ullman *et al.,* (1992) have reported the particle size of tospoviruses to be 80-110nm. Our results are in agreement with these reports. Joubert *et al.,* (1974) reported that purified TSWV preparations contained roughly spherical particles of 85 nm diameter. The present studies envelope around the core particle can be observed. The size is around 80 to 100 nm.

5.3 TRANSMISSION STUDIES

5.3.1 Mechanical transmission

Experiments on the use of buffer indicated that for sap transmission studies, 0.05M potassium phosphate buffer containing 0.02M mercaptoethanol (pH 7) was found to be better for mechanical transmission. This gave better transmission when compared to other buffer concentration evaluated. Prasad Rao *et al.* (1980) also used 0.05 M potassium phosphate buffer containing 0.02M mercaptoethanol for successful transmission. However, others used 0.1M potassium phosphate. Buffer containing 0.01M sodium sulphite (Sastry, 1982 and Krishnakumar *et al.,* 1993) for sap transmission studies. In our studies, this was less efficient as compared to 0.05 M potassium phosphate buffer with 0.02M mercaptoethanol.

Despite variable symptom expression in the field, Tospoviruses have certain unique biological properties that are useful for diagnosis they are mechanically sap transmissible as found in the present investigation to the indicator plants such as *Chenopodium amaranticolor, Petunia hybrida, D.stramonium, C. quinoa, Vigna unguiculata* (cv. C-152). This is in accordance with the reports of Best (1968); Francki and Halta (1981); De Avila *et al.* (1993b) and Nagata *et al.* (1995).

The GBNV tomato was easily detected by using indicator plants such as cowpea C-182, *D. stramonium* and immunological techniques. The results of virus detection were the similar to the earlier findings of Tospovirus detection (Lin *et al*., 1990; Hsu and Lawson, 1991 and Louro, 1996).

5.3.2 Vector transmission

In the present study, thrips species i.e. *T. palmi* and *S. dorsalis* were used. Only *T. palmi* was able to transmit the virus, whereas *S. dorsalis* failed to transmit the GBNV to tomato. It is possible that only *T. palmi* is the vector of this isolate of GBNV Tospovirus and *S. dorsalis* may not be the vector or it is also rather limited. The recent studies on vectors of peanut bud necrosis indicated thrips *T. palmi* Karny to be a potential vector of bud necrosis virus (Vijayalakshmi *et al.,* 1991). However, tospoviruses are known for erratic transmission by thrips as reported earlier by Samuel *et al.* (1930). Incompatibility between virus isolate and thrips species could be another reason for the observed non transmissibility (Paliwal, 1976). In Poland some races of *T. tabaci* were able to transmit TSWV, where as other did not and failure to transmit the virus is correlated with the absence of males in local *T. tabaci* population (Zawriska, 1976).

5.4 HOST RANGE STUDIES

Studies on development of symptoms on different hosts revealed that GBNV produces chlorotic or necrotic lesions on *Chenopodium amaranticolor, C. melo, Cucumis sativus, Citrullus lanatus and Capsicum annum, Vigna unguiculata, Luffa acutangula.* These host reactions were similar to the earlier reports of Prasad Rao *et al.,* (1980), Sastry (1982), Doraiswamy *et al.,* (1984) Sutha *et al.* (1998). The virus produced local and systemic symptoms on peanut, *D. stramonium,* tomato, and *N. glutinosa* which are accordance with the previous reports (Prasad Rao *et al.,* 1984; Sastry, 1982; Doraiswamy *et al.,* 1984). The present virus differs from WBNV as it doesn't infects cucurbits systemically whereas WBNV known to infect systemically muskmelon and watermelon (Singh and Krishna Reddy, 1996). Both tomato Tospovirus and PBNV infect cowpea cv. C-152 systemically (Ghanekar *et al.,* 1979).

5.5 MANAGEMENT OF BUD BLIGHT DISEASE

5.5.1 Screening of tomato variety/hybrid

For effective management identification of source of resistance is the best approach since insecticides to control thrips have proved largely ineffective and cultural methods to minimise the virus infection was also found to be less effective (Cho *et al.,* 1989). Of the 22

varieties hybrids screened for resistance level of incidence (10-100%) varied. None of the varieties showed immune reaction. This is similar to the earlier reports of Prasad Rao *et al.* (1980); Joi and Summanvar (1989), Jaya Jasmine and Seemanthini (1994). This may be due to innate differences among the accessions evaluated for field resistance to tomato for Tospovirus. Variation in the Tospovirus isolates was also reported to be one of the reasons for variation found in the resistance in tomato. The response of genotypes to the incidence of Tospovirus also depends on the isolate, environment (Best, 1968). Due to the Tospovirus selection pressure and evolution of resistance breaking strains, some of the tomato genotypes found resistance earlier have become susceptible (Latham and Jones, 1998).

5.5.2 Antiviral resistance

In case of the inoculated plants of cowpea (without sprays of leaf extracts) there was less activities of enzymes (PAL, PO, PPO) compared to leaf extract sprayed after 48 hours of inoculation less number of lesion development was observed, which may be due to leaf extracts sprayed may contain some inhibitory compounds like phenols in the leaf extracts which keep the host from infection plus inhibits virus multiplication if we spray plant extracts after inoculation this was observed in both coconut and sorghum leaf extracts sprayed plants. Due to induced systemic resistance, the enzymatic activity was more in inoculated and followed by leaf extract sprayed plants compare to healthy plants. Among the defense enzymes PAL activity was more in inoculated and leaf extract sprayed plants and peroxidase, polyphenol oxidase was also showed that they having same reaction. The peroxidase, PAL and PPO activity increased 2 days after inoculation followed by leaf extracts sprays that will increase defense activity through more enzyme activities.

The increased activity of the polyphenol oxidase has been reported due to either solubilization of polyphenol oxidase from cellular compartments or activation of latent polyphenol oxidase (Robb *et al.,* 1964) and also the possibility of its being released by the pathogen (Farkas *et al.,* 1962). Activity of PAL was significantly increased in leaf extract sprayed 2 days after inoculation. But in inoculated leaves showed very little change. Virus inoculation before 48 hours of leaf extracts spray and inoculation after 24 hours of leaf extract sprayed was also more enzymatic activity compared to healthy because of induced systemic resistance. In general, the activity of all these enzymes increased in inoculated and leaf extract sprayed leaves compared to unsprayed leaves. These results were supported by the some of the reports PAL, peroxidase, tyrosinase activities are known to be associated with the deposition of lignin and suberin in Plant cell (Goodman *et al.,* 1986).

5.5.3 Disease management in field

In this study, use of chemical molecules such as imidachloprid, thiomethoxam, interpid, acetamiprid, leaf extracts of coconut and sorghum and cultural practices like use of border crop like maize were evaluated to know their efficacy by keeping disease incidence at low level by controlling the vector, *Thrips palmi*, which is responsible for the spread of the disease. Seed treatment with imidachoprid (0.005%) alone was also found effective, which is on par with the border crop with maize, package of practice (seed treatment with Thiram 1.25 g) was found on par with control.

Recent emphasis on the development of non chemical control methods has given impetus to more intensive exploratory. Organic agriculture is the need of the hour. Therefore, coconut and sorghum 10 per cent leaf extracts were tried in the field. Totally 4 number of sprays were given at 15 days interval. It was found that the leaf extracts of coconut and sorghum performed better in reducing the disease incidence at an early stage. This may be due to induced systemic resistance to delay the viral multiplication and symptom expression and also may be repellent action against the vectors. The application of insecticide, imidachloprid at 0.005 per cent (T_8) at 15 days interval in the main field was found effective which kept the vector population at low level (0.91 /leaf of plant) and reduced the disease incidence (40.63%) at 80 DAT. This was still good (T_6) even when used in sequence with other chemicals like Thiomethaxam (0.05%), Acetamiprid (0.02%) and Intrepid (0.5%) which had low vector population (0.55/leaf) and low disease inc incidence (16.54%) When sprayed alternatively at 20 days interval with this seed treatment with imidachloprid (0.005%) was found best among all treatments significant difference when compared to control. Hence, the

stage of the crop at which the insecticidal treatment was given an important factor, which had direct influence on the disease incidence and vector population.

The inefficiency of chemicals to control the disease incidence may be attributed to low inoculation period, immigration of viruliferous thrips, resistance of thrips against chemicals and reduction in effectiveness of chemicals on plants over days after spraying.

FUTURE LINE OF WORK

1. Precise epidemiological studies have to be undertaken in order to avoid further spread of the disease
2. An intensive survey is needed for the identification of the prevalence of thrips species
3. New chemical molecules are needed to be tested on the thrips species
4. Field level evaluation with organics

6. SUMMARY AND CONCLUSIONS

Results of the studies are summarised below.

Natural infection of bud blight on tomato showed symptoms of chlorosis, necrosis on new growth, ring spots on leaves, yellowing, wilting, tip drying and death of plants. Some times brown necrotic streaks were observed on leaf petiole, stem and fruit stalks. Symptoms on fruits included chlorotic ring spots or discoloured and deformed fruits. Similar symptoms were recorded on tomato which were mechanically inoculated.

The survey under taken on the incidence of the bud blight on tomato in parts of Belgaum, Dharwad, Haveri and Kolar district during summer season under irrigated tomato fields revealed the presence of GBNV in all the places. The disease incidence ranged from 12.5 to 94.4 per cent and maximum was noticed in Kyalanur (94.4%) of Kolar district and minimum was in Guledkoppa (12.5%) in Dharwad taluk. Among the varieties/ hybrids surveyed, Abhinav recorded highest incidence and Utsav had relatively less incidence.

Groundnut bud necrosis virus (GBNV) was successfully transmitted by mechnical inoculation using 0.05M potassium phosphate buffer (pH 7.0) containing 0.02M mercaptoethanol. Cowpea (C-152), *Datura stramonium, Chenopodium amaranticolor. Petunia hybrida* were found to be good assay hosts.

In the host range studies, of the 28 plant species tested, 25 plant species from *Chenopodiaceae, Amaranthaceae, Fabaceae, Cucurbitaceae Asteraceae* and *Solanaceae* families were infected. Among them, 14 were found to be local lesion hosts, 9 were local systemic hosts, 2 were systemic latent hosts and 3 non hosts namely *Chrysanthemum indicum, Helianthus annuus, Pisum sativum.*

In the present investigation GBNV was transmitted by *Thrips palmi* but not by *Scirtothrips dorsalis*.

The GBNV was detected by DAC-ELISA. This method was sensitive enough to detect the virus at 1: 10000 dilution.

GBNV was amplified by RT-PCR. The coat protein gene ~830 bp cloned into a plasmid (cloning vector) to facilitate its sequencing. The comparison of sequence of CP gene of GBNV of tomato Dharwad isolate by BLAST analysis with other sequences with respect to nucleotide polymorphism revealed that the Dharwad isolate under study was 97 per cent homology with north Indian isolates of GBNV.

GBNV coat protein gene was amplified, cloned and sequenced. The sequenced region contained a single open reading frame of 831 nucleotides that could potentially code for a coat protein of 276 amino acids.

Electron microscopy studies of GBNV tomato Dharwad isolate showed the presence of spherical virus particle measuring 80-100 nm diameter.

Of the 22 tomato varieties screened for resistance under field conditions during summer 2008, infection varied from 10-100 per cent. One variety Marikrit showed moderately resistant, another cultivar NS-2535 moderately susceptible and remaining 18 were highly susceptible.

The effect of coconut and sorghum leaf extracts were studied to know the antiviral properties through ISR. There was less number of lesions production after the spray of leaf extracts 48 hours after inoculation with coconut and sorghum leaf extracts. There was incidence of 80.7 per cent by coconut and 89.5 per cent by sorghum. Spray of leaf extract before inoculation showed less enzymatic activities. Spray of leaf extracts 24 hrs after inoculation followed by reinoculation resulted in less production and activity of PAL, PO, PPO indicating that low Induced Systemic Resistance (ISR) activity. Spray of leaf extracts after 48 hours of inoculation showed high ISR activities through higher levels of PAL, PO, PPO compared to just inoculated plants.

Both the leaf extracts sprayed 48 hours after inoculation showed very high concentration of PAL, peroxidase and polyphenol oxidase. Hence, leaf extract sprayed plants showed more inhibition of lesion production and per cent incidence.

In the management of GBNV in tomato, the seed treatment with imidachloprid (0.005%) followed by spray of interpid (0.5%), acetamiprid (0.02%), thiomethoxam (0.05%) found effective with low disease incidence compared to all other treatments. The growth of tomato cv. Megha was increased with delayed GBNV infection. The plants infected within 20 days after transplanting recorded more infection. As infection delayed, there was increased growth. The fruits formed in the early infected plants were very small and mummified.

Studies based on symptomatology, host range, serological relationships using polyclonal antibodies, PCR, cloning and sequencing studies and comparison of coat protein (CP) gene sequence homology of tomato Tospovirus shared maximum sequence identity with GBNV at nucleotide (97%) and aminoacid (99%) levels. Sequence homology with GBNV of other isolates in different places indicated that the present bud blight disease caused by GBNV on tomato could be designated as groundnut bud necrosis virus infecting tomato crop.

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STUDIES ON BUD BLIGHT DISEASE OF TOMATO CAUSED BY GROUNDNUT BUD NECROSIS VIRUS

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ABSTRACT

Bud blight disease on tomato caused by groundnut bud necrosis virus (GBNV) is most important viral diseases of tomato in Karnataka and causes severe losses every year belongs to genus Tospovirus and the family *Bunyaviridae*. The survey was undertaken in parts of Belgaum, Dharwad, Haveri and Kolar districts during summer 2008. The bud blight was present in all the places where survey was carried out, the disease incidence ranged from 12.5 to 94.4%.

The GBNV was mechanically transmitted. Cowpea, Datura, Chenopodium and Petunia were found to be good assay hosts. In the host range studies, 28 plant species tested, 14 were local lesion hosts, nine were local systemic hosts, two were systemic latent hosts and three were non-hosts. The GBNV was transmitted by *Thrips palmi*. The GBNV was detected by DAC-ELISA. The GBNV coat protein gene was amplified by RT-PCR. The sequenced region contained 831 nucleotides potentially code for a coat protein of 276 amino acids. Electron microscopy studies showed spherical of 80-100 nm diameter particle size.

The effect of coconut and sorghum leaf extracts were studied to know the antiviral properties through induced systemic resistance (ISR). Spray of leaf extracts after 48 hours of inoculation, there was high ISR activities through higher production of PAL, PO, PPO compared to just inoculated plants.

Of the 22 tomato varieties screened for resistance under field conditions, none of the genotypes showed resistant reactions.

In the management of GBNV in tomato, the seed treatment with imidachloprid (0.005%) followed by spray of interpid (0.5%), acetamiprid (0.02%), thiomethoxam (0.05%) found effective with low disease incidence (34.09) compared to control (92.22). Sequence homology with GBNV of other isolates indicated that the present bud blight disease caused by GBNV on tomato can be tentatively designated as a strain of groundnut bud necrosis virus on tomato.