DISCUSSION
V. DISCUSSION

The present investigations were undertaken with the following aspects. Survey, determination of virus–transmission characters, host range, development of specific diagnostic test for the causal virus and molecular characterization of the virus by cloning and sequencing the viral genomes.

A survey was conducted during August 2011 to March 2012 in different parts of Raichur, Koppal, Tumkur, Bengaluru Rural and Bengaluru Urban districts. SuLCV disease was observed at all places of survey. The incidence of the disease varied from 0.00 to 58.00 per cent. The maximum incidence of SuLCV was observed at Main Agricultural Research, Raichur (58.00 %). The minimum incidence of 0.00 per cent was observed at thagarigunte, malangi village of Tumkur district, Vishwanathapura, Budigere, Chikkahalli and Battaramarenahalli villages of Bengaluru rural district and UAS, GKVK, Bengaluru urban district.

The differences in the incidence of disease in areas surveyed might be due to the variation in the source of inoculum, vector population, climatic conditions and the area under the crop. In recent years there have been wide spread occurrence of Begomoviruses on many crop plants as well as ornamental plant species Croton (Mahesh et al., 2010), Hibiscus (Rajeshwari et al., 2005). Some of them are also known to be source of infections for other Begomoviruses. Introduction of B-biotype whitefly has been attributed for epidemics and emergence of new viruses. The result of the study has epidemiological significance for monitoring and management of the disease. Similar observations were made by Saikia and Muniyappa (1989), Ramappa (1993).

Sunflower leaf curl virus disease causes loss by affecting growth and yield of the infected plant. In nature, SuLCV infected plants
exhibited symptoms such as vein thickening, enations on the leaves followed by upward curling of leaves, reduction in leaf lamina and stunting. Similar symptoms were observed on tomato infected with ToLCV (Saikia and Muniyappa, 1989; Seetharama Reddy, 1978).

It was observed after 20-22 days after inoculation with SuLCV, by *Bemisia tabaci*, the inoculated Sunflower seedlings produced typical vein thickening and leaf curl symptoms. In advanced stages, enations appeared all along the veins on the lower surface of the leaves. Similar symptoms also been reported in many begomoviruses infected hosts (Saikia and Muniyappa, 1989; Rangaswamy *et al*., 2005; Aswathanarayana *et al*., 2005).

The disease was transmitted through whiteflies *B. tabaci*, but the rate of transmission from Sunflower to Sunflower by whitefly was high (100%) with 20 whiteflies. Transmission of SuLCV from Sunflower to Sunflower resulted in 40.00 per cent success with 5 adult whiteflies per plant. Low transmissibility of begomovirus associated with woody plant species like cassava (ICMV) and hibiscus (HbLCV) by whiteflies was reported by earlier workers Mathew (1988). Transmission of ICMV from and to cassava was reported to be only 18.7 per cent and that of HbLCV from and to hibiscus, only 20 per cent with 50 and 25 whiteflies per plant respectively (Rajeshwari *et al*., 2005).

The studies on virus-vector relationship, single whitefly transmitted SuLCV to an extent of 10 per cent. However, 5 or more whiteflies were required for 100 per cent transmission. There existed a positive correlation between the number of whiteflies and SLCV transmission. This finding is in confirming with other whitefly transmitted geminiviruses.
Host range studies revealed that the virus was limited to the only few plant species belonging to Solanaceae and Asteraceae. Out of 12 plant species inoculated, two plant species belonging to Solanaceae were infected with SuLCV viz., *N. tabacum, L. esculentum*. Three plant species, *Zinnia elegans, Acanthospermum hispidum* and *Parthenium hysterophorus* belongs to Asteraceae was infected with SuLCV through whiteflies and expressed the systemic symptoms within 30 days. Similar host ranges recorded by Seetharama Reddy, 1978; Sastry, 1984 in ToLCV transmitted plants.

In the present study, Sunflower leaf curl virus was detected in the Sunflower by PCR tests using five sets of primers (Deng, *et al.*, 1994), (Wyatt and Brown, 1996), (Chowda Reddy, *et al.*, 2005), (Briddon *et al.*, 2002) and (Bull, *et al.*, 2003) amplified expected PCR products of size ~520 bp, ~575 bp, ~2.8 kb, ~1.3 kb and ~1.3 kb respectively. Primers designed to amplify the conserved region of the CP gene, DNA-A component and satellite molecules beta and alpha employed by many workers to identify and confirm the diseases caused by begomoviruses in wide range of crop plants.

The CP acts as the coat of the virus particle and is essential for virus transmission from diseased to healthy plants by *B. tabaci*. The CP is highly conserved amongst the begomoviruses originating from the same geographical region and thus been adapted to transmission by local vector populations (McGrath and Harrison, 1995; Mandal and Muniyappa, 1991; Maruthi *et al.*, 2002a; Shankarappa, 2006). The CP is therefore, an essential component of begomoviruses and has been used widely to characterise and establish the relationship of many begomoviruses (Harrison *et al.*, 2002).

PCR test was employed and amplified PCR products of size ~520 bp and ~575 bp core region of CP gene of SuLCV, from Sunflower
samples using two sets of degenerate primer (Deng, et al., 1994), (Wyatt and Brown, 1996). These primers used elsewhere, successfully detected and Croton leaf curl virus (CrLCuV) diseases from infected plants, Zinnia leaf curl virus (ZLCV) (Shivakumar, 2010), Hibiscus leaf curl disease (HLCuD) (Rajeshwari et al., 2005) and Jatropha Mosaic Virus (Rangaswamy et al., 2005; Aswathanarayana et al., 2007) Tomato leaf curl virus (Shankarappa et al., 2007).

The symptoms of SuLCV closely resembles the symptoms of ToLCKV and shared highest coat protein nucleotide identity (97.5%). Thus appears that the SuLCV is a distinct begomovirus closely related to ToLCKV-(Luc) and ToLCBV-(Ban2). This finding is in conformity with study conducted on Zinnia leaf curl virus, the core CP nucleotide sequences of ZLCV were compared with those of selected begomoviruses obtained from the NCBI database. Phylogenetic analysis of the sequences grouped ZLCV-[Ban] in separate cluster next to the tomato leaf curl virus Bangalore isolate-II (ToLCV-Iso II) and tomato leaf curl Karnataka virus-Lucknow (ToLCKV-[Luc.]). They were 97 per cent similar to each other and shared the highest nucleotide identity of 97 per cent with both ToLCV-[Luc] and ToLCKV-[BanII].

The association of begomovirus with Sunflower leaf curl virus disease was confirmed by cloning and sequencing of the full genome of the virus. SuLCV was confirmed with the sequence information of approximately 2761 bp fragment amplified using Full genome specific primers (DNA-A) capable of amplifying monopartite begomoviruses. These results are in conformity by molecular study of monopartite tomato leaf curl virus in India (Chowda Reddy, et al., 2005).

The full genome nucleotide sequences of ToLCKV (Raichure: Sunflower) was compared with those of selected begomoviruses obtained from the NCBI database. Phylogenetic analysis of the sequences grouped
ToLCKV (Raichure: Sunflower) in a ToLCKV [IKH12] with 97.13% sequence similarity followed by 96.95% sequence similarity with ToLCKV-[IKB3] and 95.65% with ToLCV-[Ban-II] (U38239) respectively. The results are in conformity with the study conducted on cloning, sequencing and phylogenetic analysis of DNA-A component of Tomato leaf curl Gujarat virus causing leaf curl disease on tomato indicates that it consist of 2,757 bp nucleotide length and phylogenetic analysis placed ToLCGV-[Var] in a unique cluster with two other isolates (97% and 99%) ToLCGV [Vad] from Vadodara and ToLCGV [Mir] from Mirzapur. The other closest tomato infecting Indian geminivirus is ToLCKV with 84% nucleotide identity (Chakraborty, et al., 2003).

The DNA-A of the Begomovirus associated with TbLCD in Pusa, Bihar was found to comprise of 2,707 nt with a typical Old World begomovirus-like genome organization. The full-length sequence of DNA-A [HQ180391] showed that the Pusa isolate is a newly described member of the genus Begomovirus, as it had <89% sequence homology with DNA-A of all the known begomoviruses. The isolate is tentatively named as Tobacco leaf curl Pusa virus [India:Pusa:2010] (Singh, et al., 2011).

The Hibicus leaf curl virus was characterised by sequencing a fragment of DNA-A component (1288 nucleotides). Phylogenetic analyses of these DNA-A sequences clustered them with Old World cotton-infecting begomoviruses and closest to Cotton leaf curl Multan virus (CLCuMV) at 95–97% DNA-A nucleotide identities (Rajeshwari, et al., 2005).

The full-length DNA beta satellite was detected through PCR and the sequence was determined to be 1,373 nt. Sequence analysis demonstrated that in phylogenetic analysis of beta satellite DNA associated with SuLCD branch with Potato apical leaf curl disease-associated satellite DNA beta PaLCuB-[IN:Chi:03] with (94.07%)
nucleotide identity and clustered with some of the other betasatellites molecules associated with begomovirus causing disease of tomato and potato occurring in India. Similar results was obtained by Briddon et al. (2002) where PCR detection of full length beta satellite molecule was carried out using beta 01 and beta 02 primer pair was successfully amplified 1350 bp in Cotton leaf curl virus (Briddon et al., 2002)

The PCR amplification of ~1.3 kb was obtained with betasatellite DNA molecule of sunn hemp leaf curl virus and further nucleotide sequence analysis showed sequence similarity with previously characterized betasatellite DNA of begomoviruses affecting Malvaceous crops from different regions of India and Pakistan. Maximum similarity (90%) of betasatellite DNA under study was observed with Cotton leaf curl Multan betasatellite (CLCuMB-Pak: Mul17:08) and other betasatellite DNA from Pakistan (Kumar et al., 2010).

The alpha DNA satellite molecule was amplified through PCR using specific primers and the cloning work was carried out. The sequence consisted of 1,350 nucleotides. In phylogenetic analysis of ToLCKV (Raichure: SF) alpha satellite DNA associated with SuLCD branch with Tobacco curly shoot alphasatellite affecting wild Sunflower in India and clustered with some of the alphasatellites molecules of other begomoviruses causing disease of tomato and tobacco occurring in the India and China. The PCR detection studies are in conformity with study conducted by Bull, et al. (2003) used UN 101 and UN 102 primers to obtain amplification of ~1.3 kb from Cotton leaf curl disease associated DNA 1 (CLCuD DNA 1).

Cloning and sequencing result of SuLCV alpha satellite molecule are in conformity with findings on a monopartite Begomovirus, alphasatellite were found associated with the leaf curl disease of tobacco (TbLCD) disease in Pusa, Bihar. The alphasatellite (HQ180392)
associated with the disease had highest 87% nucleotide sequence identity with Tomato leaf curl alphasatellite. The *Begomovirus*, betasatellite, and alphasatellite associated with TbLCD in Pusa, Bihar, India were found to be recombinants of extant begomoviruses, betasatellites and alphasatellites spreading in the Indian sub-continent and South-East Asia (Singh et al., 2011).

Based on symptomatology, transmission studies and successful detection of the virus by PCR using four sets of primers, and the similarity of the sequences of full genome, beta and alpha satellite molecules with other begomoviruses, it is concluded that the virus causing leaf curl disease in sunflower is a new begomovirus and is probably caused by ToLCKV and detection of α-satellite and β-satellite DNA from SuLCV infected plants clearly indicates that the virus is a Begomovirus with monopartite genome.

**Future line of work**

1. Genetic diversity among the isolates of SuLCV from different geographical area
2. Development of Infectious clones of the virus
3. Development of transgenic sunflower plants against sunflower leaf curl virus
4. Detailed investigation on epidemiology, screening of available germplasm for their resistance to the virus and development of suitable management methods for the disease.