DISCUSSION

Genus *Malus* originated in Europe but its species are now cultivated worldwide. Many cultivars of *Malus*, the most important of these species are used as rootstock. Virginia crab is especially important as it is mainly used as rootstock. Many fungal, bacterial and viral diseases cause economic losses to Apple fruit. Among these viral diseases ASGV is of huge concern to both farmers and scientists due to severe affect on growth and quality of fruit.

Viral symptomatology:

Identification and characterization of virus are the first steps in virus management. Infected plant usually shows sunken areas and longitudinal grooves. Strain E-36 causes graft union to be brown, fragile, swollen and sunken area on stem (Lister et al1965)). The virus symptoms on plant were observed in young stage of plant. In the field plants was seen healthy, however sometimes contain viruses. Young leaf samples were collected and tested for presence of ASGV. Through serological & biological methods, RT-PCR.

Serological (DAS-ELISA) detection

Though serology is a very useful criterion for the identification and classification of several plant virus groups (Van Regenmortel, 1982), it has proved most unsatisfactory against viruses of the Family Flexiviridae due to inherent complexities associated with coat proteins and particles. In spite of these polyclonal antisera have been widely used in serological detection of ASGV.

Since the development of ELISA, it is one of the most popularly used

techniques till today for detection of viruses. ASGV is not highly immunogenic, so serological method of detection cannot be reliable method for its detection. ELISA has been found to be a best way for large scale routine testing of viruses due to its rapid, cost effective, simple and fast assay properties. According to the proposed plan DAS-ELISA was performed to check the presence of virus in 6 plants. Each plant was tested at fully opened mature leaf stage. Out of the 6 plants tested 1 plant (Fr-3) showed positive result indicating the viral infection.

RT-PCR based detection

Limitations of ELISA to detect virus may be overcome by the use of RT-PCR technique, which is more sensitive than ELISA and can detect as low as 50-200 ng concentration of virus (Kinard *et al.,* 1994). In the present study, out of 2 samples were tested & were found positive for ASGV. The numbers of samples found positive for ASGV were more through RT-PCR in comparison to ELISA. The results of RT-PCR showed that some plants which are not positive for DAS-ELISA were found positive through RT-PCR, showing that RT-PCR is more reliable and sensitive than ELISA based detection. Similar results were also reported by Candresse *et al.* (2000).

A set of oligonucleotide primers were designed on the basis of conserved sequences present in the genome on either side of coat protein gene. The conditions for RT-PCR, PCR were standardized by checking different temperatures around annealing temperature of primers and a temperature of 60^{o}C was found suitable for proper amplification of ASGV coat protein gene. PCR results in amplification of ~300 bp DNA fragment which includes only a small part of complete coat protein gene The reliability of this method was

demonstrated earlier by Nemchinov *et al.* (1995).

Cloning and sequencing of coat protein gene

The ampilied PCR product was cloned into pGEMT easy vector .Positive Cloning were identified by Restriction Digestion by *EcoR 1* and Pure Plasmid were isolated and 200ng of Plasmid was given to Genei Banglore for sequencing.