CHAPTER

29

Virus Diseases of Groundnut

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ABSTRACT

Groundnut or peanut is an important annual oilseed and food legume crop grown in diverse environments throughout the tropical world. About 31 viruses representing 14 genera were reported to naturally infect groundnut in different parts of the world. Among these, *Tomato spotted wilt virus* (TSWV), *Groundnut bud necrosis virus* (GBNV), *Tobacco streak virus* (TSV), *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV), satellite RNA associated with GRV and/or GRAV, *Peanut clump virus* (PCV), *Peanut stripe virus* (PStV), a strain of *Bean common mosaic virus* (BCMV), *Peanut mottle virus* (PeMoV) and *Cucumber mosaic virus* (CMV) have either global / regional economic importance. *Cowpea aphid-borne mosaic virus* (CABMV), CMV, IPCV, PCV, PeMoV, PStV, PSV and *Cowpea mild mottle virus* (CPMMV-severe strain) are transmitted through groundnut seed and thus are important quarantine pests. A review on the distribution, incidence, symptoms, impact on crop growth and yield, virus properties, phylogenetic relationships, virus detection, epidemiology and management of TSWV, GBNV, TSV, rosette complex, peanut clump, PStV, PeMoV and CMV, is presented in this chapter.

Key Words: Groundnut, spotted wilt, bud necrosis, stem necrosis, groundnut rosette, peanut clump, peanut mottle, peanut stripe, yellow mosaic.

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1. INTRODUCTION

Groundnut or peanut (*Arachis hypogaea* L.) native to South America is an annual oilseed and food legume crop grown in diverse environments throughout the world between 40°N and 40°S. Presently it is cultivated in 26.3 million ha with productivity of 36 million Mt (FAOSTAT data, 2004). The most important groundnut producing countries are Argentina, Chad, Ghana, China, India, Indonesia, Myanmar, Nigeria, Senegal, Sudan, USA and Vietnam. Groundnut seed contains about 50% edible oil and 25% protein and is consumed as whole seed or processed as snack foods. Groundnut oil is used for cooking and the residual cake is used in animal feeds. The haulms are used as hay for feeding livestock. Nearly 75 to 80% of world's groundnut is grown by resource poor smallholder farmers in developing countries who routinely obtain yields of 500-800 kg ha^{-1} as opposed to the potential yields of >2.5 t ha^{-1}. Low yields are mainly due to numerous diseases caused by fungi, viruses, bacteria and nematodes (Kokalis Burelle *et al*., 1997; McDonald *et al*., 1998). Of these, diseases caused by viruses are most important. About 31 viruses were reported to naturally infect groundnut around the world (Table. 1). Nine of them belong to genus *Potyvirus,* six to *Tospovirus,* two each to *Cucumovirus*, *Pecluvirus*, *Soymovirus* and *Umbravirus,* and one each to *Begomovirus, Bromovirus, Carlavirus, Ilarvirus, Luteovirus, Potexvirus, Rhabdovirus* and *Tymovirus.* Of these, 19 were first isolated from groundnut, and remaining first isolated from other hosts but they commonly occur on groundnut. Diseases in groundnut caused by *Cucumber mosaic virus* (CMV), *Groundnut bud necrosis virus* (GBNV)*, Groundnut rosette virus* (GRV), *Indian peanut clump virus* (IPCV), *Peanut clump virus* (PCV), *Peanut mottle virus* (PeMoV), *Peanut stripe virus* (PStV)*, Tobacco streak virus* (TSV) *and Tomato spotted wilt virus* (TSWV) are most economically important and are responsible for serious yield losses globally or regionally. They also known to naturally infect several other crops and inflict significant losses in them. In this chapter these nine viruses were discussed in detail. Eight groundnut-infecting viruses, CMV, IPCV, PeMoV, PStV, PCV, *Cowpea mild mottle* - severe strain (CPPMV), *Cowpea aphid-borne mosaic viruses* (CABMV) and *Peanut stunt virus* (PSV) are seed-transmitted and they have quarantine importance (Sivaprasad and Sreenivasulu, 1996; Demski and Reddy, 1997; McDonald *et al.,* 1998; Pio-Ribeiro *et al.,* 2000; Reddy and Devi, 2003).

2. *TOMATO SPOTTED WILT VIRUS* (SPOTTED WILT)

Spotted wilt caused by TSWV is the type species of the genus *Tospovirus,* family *Bunyaviridae,* whose members also infect various invertebrates and vertebrates (Van Regenmortel *et al.,* 2000). It was first recognized in tomatoes in Australia in 1915 (Brittlebank, 1919). The

Table. 1: Distribution and characteristics of certain viruses reported to naturally infect groundnut

Virus species [Synonyms]	Genus (Family)	Distribution	Transmission	References
Bean golden yellow mosaic virus (BGYMV) [Peanut yellow mosaic virus]	*Begomovirus (Geminiviridae)*	India	Whiteflies	Sudhakar Rao *et al.*, 1980
Bean yellow mosaic virus (BYMV)	*Potyvirus (Potyviridae)*	United States	Sap, aphids (NP)	Bays and Demski, 1986
Cowpea aphid-borne mosaic virus (CABMV)	*Potyvirus (Potyviridae)*	Brazil	Sap, seed (<1 %), aphids (NP)	Pio-Ribeiro *et al.*, 2000
Cowpea chlorotic mottle virus (CCMV)	*Bromovirus (Bromoviridae)*	USA	Sap, beetles	Kuhn and Demski, 1987
Cowpea mild mottle virus (CPMMV) [Groundnut crinkle virus]	*Carlavirus (Flexiviridae)*	China, India, Indonesia, Ivory Coast, Nigeria, Thailand, Philippines, Papua New Guinea, Sudan	Sap, whiteflies, seed	Dubern and Dollet, 1981 Iizuka *et al.*, 1984 Sivaprasad and Sreenivasulu, 1996.
Cucumber mosaic virus (CMV)	*Cucumovirus (Bromoviridae)*	*China*	Sap, aphids (NP), seed (2-4%)	Xu and Barnett, 1984
Groundnut bud necrosis virus (GBNV) [Peanut bud necrosis virus]	*Tospovirus (Bunyaviridae)*	India, Nepal, Sri Lanka, China, Taiwan, Indonesia, Thailand	Sap, thrips (CP)	Ghanekar *et al.*, 1979; Reddy *et al.*, 1992
Groundnut chlorotic spotting virus (GCSV)	*Potexvirus*	Ivory Coast	Sap	Fauquet *et al.*, 1985; Dollet *et al.*, 1987
Groundnut chlorotic fan -spot virus (GCFSV)	*Tospovirus (Bunyaviridae)*	Taiwan	Sap, thrips (CP)	Chu *et al.*, 2001
Groundnut eyespot virus (GEV)	*Potyvirus (Potyviridae)*	Ivory Coast	Sap, aphids (NP)	Dubern and Dollet, 1980

Virus species [Synonyms]	Genus (Family)	Distribution	Transmission	References
Groundnut rosette assistor virus (GRAV)	*Luteovirus (Luteoviridae)*	All of Africa, south of the Sahara	Aphids (C)	Hull and Adams, 1968; Reddy *et a1.*,1985a; Rajeshwari and Murant, 1988; Naidu *et al.*, 1999a
Groundnut rosette virus (GRV)	*Umbravirus*	All of Africa, south of the Sahara	Sap, aphids (helper-dependent transmission)	Naidu *et al.*, 1999a; Taliansky *et al.*, 2000.
Groundnut ring spot virus (GRSV)	*Tospovirus (Bunyaviridae)*	South America, Africa	Sap, thrips (CP)	De Avila *et al.*, 1993b; Peters, 2003.
Groundnut veinal chlorosis virus (GVCV)	*Rhabdovirus (Unassigned)*	India, Indonesia	Ni	Naidu *et al.*, 1989
Ground yellow spot virus (GYSV) [Peanut yellow spot virus]	*Tospovirus (Bunyaviridae)*	India, Thailand	Sap, thrips (CP)	Wongkaew, 1986; Reddy *et al.*, 1991; Satyanarayana *et al.*, 1996a.
Impatiens necrotic spot virus (INSV)	*Tospovirus (Bunyaviridae)*	United States	Sap, thrips (C)	Pappu *et al.*, 1999
Indian peanut clump virus (IPCV)	*Pecluvirus*	India, Pakistan	Sap, plasmodiophoromycete fungi, seed (up to 6%)	Reddy *et al.*,1983b; Nolt *et al.*,1988; Reddy *et al.*, 1999
Peanut chlorotic streak virus (PCSV)	*Soymovirus (Caulimoviridae)*	India	Sap	Reddy *et al.*, 1993
Peanut chlorotic streak virus – Vein Banding Strain (PCSV-VB)	*Soymovirus (Caulimoviridae)*	India	Sap	Satyanarayana *et al.*, 1994a; 1994b.
Peanut chlorotic blotch virus (PCBV)	*Potyvirus (Potyviridae)*	South Africa	Sap, aphids (NP)	Cook *et al.*, 1998
Peanut clump virus (PCV)	*Pecluvirus*	Niger, Burkina Faso, Ivory Coast, Senegal,	Sap, plasmodiophoromycete fungi, seed (up to 6%)	Thouvenel *et al.*, 1976; Reddy *et al.*, 1999
Peanut green mosaic virus (PGMV)	*Potyvirus (Potyviridae)*	India	Sap, aphids (NP)	Sreenivasulu *et a1.*, 1981

Virus species [Synonyms]	Genus (Family)	Distribution	Transmission	References
Peanut green mottle virus (PeGMoV)	*Potyvirus (Potyviridae)*	ni	ni	http://www.danforthcenter.org/iltab/potyviridae/descriptionfiles/references.html
Peanut mottle virus (PeMoV) [Peanut mild mosaic] [Peanut severe mosaic] [Groundnut mottle virus]	*Potyvirus (Potyviridae)*	Worldwide	Sap, aphids (NP), seed (up to 8.5%)	Kuhn, 1965; Behncken, 1980; Bock 1973; Paguio and Kuhn, 1973; Rajeshwari *et a1.*, 1983
Peanut stripe virus (PStV)	*Potyvirus (Potyviridae)*	Brazil, China, India, Indonesia, Japan, Malaysia, Philippines, Myanmar, Thailand, Taiwan, Vietnam, United States	Sap, aphids (NP), seed (up to 35%)	Demski *et a1.*,1984; 1988; Xu *et a1.*, 1983
Peanut stunt virus (PSV)	*Cucumovirus (Bromoviridae)*	Sudan, Japan, Spain, United States	Sap, aphid (NP), seed (up to 5%)	Miller and Troutman, 1966; Fisher and Lockhart, 1978
Peanut yellow mottle virus (PeYMV)	*Tymovirus*	Nigeria	Sap, beetle	Lana, 1980
Passion fruit woodiness virus (PWV)	*Potyvirus (Potyviridae)*	Australia	Sap, aphids (NP)	Boswell and Gibbs, 1983
Sunflower yellow blotch virus (SuYBV)	*Umbravirus*	Malawi, Kenya, Zambia, Tanzania	Sap, aphids (helper-dependent transmission)	Theuri *et al.*, 1987
Tobacco streak virus (TSV)	*Ilarvirus (Bromoviridae)*	Brazil, India	Sap, pollen	Costa and Carvalho 1961; Prasada Rao *et al.*, 2003[b]
Tomato spotted wilt virus (TSWV)	*Tospovirus (Bunyaviridae)*	North America, South America, Nigeria	Sap, thrips (CP)	Culbreath *et al.*, 2003 (review)

NP – non-persistent transmission; C – circulative transmission; CP – circulative and propagative transmission; Ni – no information available

involvement of thrips in transmission of the causal agent was reported in 1927 (Pittman, 1927). Since then TSWV was recognized on >800 species of flowering plants from over 82 families (German *et al.,* 1992; Peters and Goldbach, 1995; Ullman *et al.,* 2002; Peters, 1998, 2003). TSWV occurrence on groundnut was first reported in Brazil (Costa, 1941). Subsequently spotted wilt or similar diseases in groundnut were reported from North and South Americas (Argentina, Brazil and USA), several African countries (South Africa, Nigeria, Kenya, Malawi and Uganda) and Australia (Cullbreath *et al.,* 2003). Its occurrence in Asia was also reported, but subsequent studies showed that it is a distinct tospovirus, now regarded as GBNV (*Peanut bud necrosis virus*) (Reddy *et al.*, 1992). Besides TSWV and GBNV, several tospoviruses like *Peanut yellow spot virus* (PYSV), *Groundnut ring spot virus* (GRSV), *Impatiens necrosis virus* (INSV) and *Peanut chlorotic fan spot virus* (PCFV) also occur in groundnut. *Watermelon bud necrosis virus* (WBMV) was shown to infect groundnut by experimental inoculation (Jain *et al.,* 1998), but there are no reports of its natural occurrence on groundnut.

Diseases caused by TSWV have been reported under various names in groundnut, lettuce, peas, pepper, potato, tobacco, tomato, papaya, pineapple and various other plant species of agricultural and horticultural importance in different geographical regions (Peters, 2003). However, in recent years several previously regarded strains of TSWV were classified as distinct tospovirus species. TSWV is vectored by several species of thrips (*Thrips tabaci, T. palmi, T. setosus, Frankliniella* spp., *Scirtothrips* spp.) in a circulative and propagative (multiply in the vector) manner, but the virus is not transmitted through seed or pollen (Mandal *et al.*, 2001; Peters, 2003). The virus can be transmitted by mechanical sap inoculation but with difficulty due to low virus concentration and virus instability in sap extracts. The virus produces a wide range of symptoms from local lesions (chlorotic and/or necrotic) to systemic symptoms (stunting, systemic necrosis, systemic wilt, spots, streaking, mosaic, mottling, leaf distortion, vein yellowing, ring spots, line patterns, yellow netting and flower color breaking) on different susceptible plant species. However, in some plants like dahlia and chrysanthemum the virus causes latent infection. Losses up to 100% have been reported due to TSWV (Cullbreath *et al.*, 2003)

The virions of TSWV and other tospoviruses are roughly spherical or pleomorphic with a diameter of 80-110 nm. The virions contain three pseudo-circular nucleocapsids packed within a lipid membrane that has surface projections (spikes) probably consisting of two glycoproteins (G1 and G2). The genome is tripartite, linear, ambisense ssRNA encapsidated by the nucleocapsid protein (N), with which a few RNA polymerase (transcriptase) protein molecules are also associated. The largest RNA molecule (L, 8897 nts) is of negative polarity and encodes 332-kDa protein, referred as the L protein that represents viral transcriptase. The M (medium)

and S (short) have ambisense polarity, each representing two genes arranged in opposite orientation. The M RNA (4821 nts) in the viral complementary sense encodes 127.4 kDa precursor to the G1 and G2 proteins, while it encodes non-structural (NSm, 33.6 KDa) protein in the viral sense. Similarly, the S RNA (2961 nts) encodes for two genes, N protein (28.8 kDa) in its viral complementary sense and a non-structural protein (NSs, 52.4 kDa) in the viral sense. The M and S RNA segments are translationally expressed via two sub-genomic RNAs (Peters and Goldbach, 1995; Peters, 2003).

2.1 Symptoms and Cytopathology

TSWV causes a variety of symptoms on peanut viz. concentric ring spots, various patterns of chlorosis on leaflets, stunting, small and mis-shaped geocarpophores (pegs), pods and kernels and discoloration and cracking of the seed coats. Symptoms range in severity from minor spotting on few leaflets to severe stunting and death of entire plant. The disease reduces the number of pods produced, kernel size and yield per plant, and these effects have been correlated with the time of initial infection. The virus has been associated with general chlorosis and wilting of peanut plants, sometimes may not be accompanied by typical above ground symptoms of spotted wilt. Roots of affected plants exhibit varying degrees of necrosis, which can result in death of entire plant. Other pathogens may also involve in the destruction of root systems of the infected plants. Asymptomatic infections were also reported based upon immunoasssays (Demski and Reddy, 1997; Cullbreath *et al.,* 2003).

TSWV and other tospoviruses exhibit similar cytopathic effects in infected cells and tissues (Kitajima *et al*., 1992). The enveloped virions are usually observed within the membrane bound cavities of the endoplasmic reticulum of thin sections. The particles of some isolates are arranged in crystalline arrays within these cavities, while a few isolates disperse their virus particles individually or in very low numbers in separate vesicles with distinct membranes. Virus particles are found in leaf cell types like trichomes, epidermis, mesophyll and vascular parenchyma cells. They also have been found in differentiating xylem vessels but not in sieve tubes. A common feature of infected cells is the presence of amorphous, moderately stained dense material, referred as viroplasm (30 – 120 nm dia and consists of rough cubic, circular or elliptic fibers). Immunolabelling with N antibodies revealed that viroplasms consist of N protein, and these are considered to be aggregates of non-encapsidated nucleocapsids. Inclusions with filamentous materials were also found in tissues infected with several isolates. The flexuous filaments (10 nm thick) form inclusions in which they are packed either in an unarranged way or in parallel arrays. So in cross sections they could be seen as crisscrossed paracrystalline arrays or

as a series of dots. These filaments contain NSs protein. The abundance of these filamentous inclusions in thin sections varies with virus isolates and host tissues (Peters and Goldbach, 1995).

2.2 Molecular diagnosis

Field diagnosis of tospoviral infections based on symptoms may mislead as they induce variety of symptoms, which are sometimes influenced by environment. Bioassays involving sap inoculation of suspected plant samples for TSWV to diagnostic hosts like *Nicotiana glutinosa, Vinca rosea, Petunia hybrida, Vigna unguiculata* and *Trapaeolum majus* have been used. But this approach has limitations like unsuitability for large scale testing, requires longer time and difficulty in the persistent sap inoculation procedures. But bioassays are still useful in simple laboratories for the diagnosis of the TSWV. Peters (2003) has compared the reaction of several frequently used plant species to sap inoculation of several tospoviruses. This information could be useful in the detection, separation and identification of tospoviruses. Another approach is the transmission of the virus by thrips to indicator hosts, but again this is unsuitable for large-scale and routine application.

Electron microscopy-based identification of tospoviruses in leaf-dip preparations has been used (Kitajima, 1965; Ie, 1971). But this method has limitations for large-scale applications and sometimes virus particles appear like host-membrane bodies and vice versa, complicating the detection.

Development of immunodiagnostics for TSWV was hampered for a long time due to difficulties in the virus purification. Subsequent establishment of good protocols for successful purification of the virus led to the production of polyclonal antiserum to nucleocapsid protein particles of TSWV. Later monoclonal antibodies and antibodies to recombinant viral protein of some isolates of TSWV were produced (Bandla *et al.*, 1994). Using these antibodies different ELISA formats and immunological squash blots have been optimized for routine large-scale detection of TSWV for testing in plant tissues and also in thrips (Gonsalves and Trujillo, 1986; Huguenot *et al.*, 1990; Resende *et al.*, 1991). The ELISA and Western analysis techniques have also been used to determine the antigenic relationships among the tospovirus isolates (Sreenivasulu *et al.*, 1991a; Adam *et al.*, 1993).

Riboprobes and cDNA probes have been developed for sensitive detection of TSWV isolates by dot-blot hybridization (Ronco *et al.*, 1989; Huguenot *et al.*, 1990; Rice *et al.*, 1990). The hybridization techniques are not widely used because they are only as sensitive as ELISA, high costs and the need for specialized facilities. The genome sequence data of the virus has facilitated to design oligonucleotide primers (universal primers, species specific primers) for RT-PCR detection of TSWV and other tospoviruses (Weeks *et al.*, 1996; Jain *et al.*, 1997; Jain *et al.*, 1998). To

improve the sensitivity of RT-PCR, immunocapture of viral particles step has been introduced (IC-RT-PCR). PCR techniques have been successfully employed for detection of virus not only in plant samples but also in thrips vectors (Jain *et al.,* 1997; Jain *et al.,* 1998). Real-time fluorescent RT-PCR was used for the detection of TSWV in individual thrips (Boonham *et al.,* 2002). The advanced nucleic acid based detection techniques require well-developed laboratory facilities and expensive. Because of this antibody-based TSWV detection assays are more popularly used worldwide.

2.3 Phylogenetic relationships

Large taxonomic differences anticipated among various isolates of TSWV occurring worldwide due to its ability to infect diverse plant species, its transmission by different thrips species and its occurrence in different geographical regions. Initial attempts were made to classify TSWV isolates into three groups based on host responses (Norris, 1946). Later, Best and Gallus (1955) distinguished six strains by symptom expression on three indicator hosts. This virus was considered as a single species under monotypic plant virus group for a long period due to lack of methods for biochemical characterization of various isolates. The application of ELISA and Western blot analysis paved the way to differentiate various isolates and to cluster them into serogroups (De Avila *et al.,* 1990; Sreenivasulu *et al.,* 1991a; Adam *et al.,* 1993). Analysis of different isolates by polyclonal antisera produced against the nucleocapsid proteins and a panel of monoclonal antibodies to N protein and to the glycoproteins resulted in the recognition of three serogroups (De Avila, *et al.*, 1990). Two serotypes in serogroup II have been established based on reactions with monoclonal antibodies to the glycoproteins. Later five serogroups represented by different viruses have been recognized in the genus *Tospovirus*: I (TSWV); II (TSWV-B, GRSV, *Tomato chlorotic spot virus*); III (INSV); IV (GBNV, *Watermelon silver mottle virus* (WSMV); V – PYSV (Satyanarayana *et al.,* 1998).

The relationships between the isolates of different serogroups were further studied by molecular hybridization and nucleotide sequence comparison (De Avila *et al.,* 1993a, 1993b; Satyanarayana *et al.*, 1998; Moyer, 1999). Analysis of the sequences of N and NSs proteins of different tospoviruses has lead to their grouping into five serogroups represented by different tospoviruses. Viruses with amino acid identity lower than 90% are considered to be different species. The members of five serogroups also exhibit differences in host range, thrips transmission and serological cross-reaction.

2.4 Epidemiology and Management

Cullbreath *et al.*, (2003) have extensively reviewed the epidemiology and management of spotted wilt disease of groundnut in the USA. Transmission of TSWV by viruliferous thrips appears to be the only means of inoculation in natural epidemics. *Frankliniella fusca, F. occidentalis* (western flower thrips), *F. intonsa* and *F. schultzei* are confirmed vectors of TSWV, and both the species occur in most groundnut growing areas in Northern America. *Thrips tabaci* is also a vector of TSWV but its role has not been implicated in the spread of TSWV in groundnut. Several sources of vectors and TSWV inoculum have been identified but their relative importance is yet to be understood. Since the virus is acquired at only larvae stage, the virus source plant must support the reproduction of vector species. Limited studies on progress of spotted wilt disease in peanut in the United States indicate that most infections are the result of primary spread and there is only limited secondary spread of TSWV after it becomes established in the field. Disease progress in groundnut has not been correlated directly with thrips population or damage caused by thrips larvae on leaves. As no single management practice provides adequate control of the disease in groundnut, a multidisciplinary and multifaceted and extension approach has been employed to improve management of spotted wilt of groundnut

Field resistance and cultivar selection: The single most important factor in management of this disease is cultivar selection. Groundnut cultivars with moderate levels of field resistance to the virus are available and widely planted in the southeastern United States. Intensive screening of cultivars and breeding lines in Georgia has led to the identification and release of moderately resistant runner type cultivars (Georgia Browne, Georgia Green, UF MDR 98, Tamrun 96 and ViruGard). All these cultivars have field resistance similar to Southern Runner (Cullbreath *et al.*, 2003). The cultivar C-99R is found to have a higher level of resistance than Southern Runner or Georgia Green. The mechanism responsible for the field resistance in peanut breeding lines has not been elucidated. The reduced incidence of disease in these lines appears to be due to reduced attractiveness to thrips vectors, reproduction of vector thrips or physical injury caused directly by thrips feeding. USDA-ARS breeding line C11-2-39 has resistance to TSWV based on mechanical inoculation studies as well as field resistance. Most of the lines with greater levels of field resistance to TSWV require longer period (~145-150 days) to mature than Georgia Green that requires 135 days.

Cultural practices and chemical control: Manipulation of planting date is a viable practice to suppress spotted wilt. Optimum planting date to minimize the disease vary among years and locations. Location of groundnut fields relative to earlier planting can be a significant factor as planting new crop down-wind from earlier planted groundnut may have

increased risks of damage by spotted wilt. Infection of groundnut plants with virus is greater among sparse plant populations than among dense populations. Twin row planting of irrigated groundnut is shown to have lower incidence of the disease, higher yields and improved grades.

Insecticidal control of thrips vectors is largely ineffective for suppressing spotted wilt in groundnut (Cullbreath *et al.*, 2003). Use of some insecticides (imidacloprid) found to increase the incidence. Aldicarb, acephate and carbofuran were found to be ineffective. Chlorophyrifos and phorate (furrow application) reduced the spotted wilt in groundnut. Herbicides have been reported to have both positive and negative effects on spotted wilt manifestation in groundnut.

Integrated management: Multiple factors have to be integrated to minimize the effects of spotted wilt in groundnut. For example, TSWV losses can be reduced by adapting combinations of two or more of the following: (a) cultivation of moderately resistant cultivars; (b) delay in planting date of early to mid-late May; (c) establishing higher plant populations; (d) adopting phorate insecticide applications; (e) planting groundnut seed in twin-rows. Weeds and volunteer groundnut serve as hosts of TSWV and/or thrips vector, and removal of such plants contribute to reduction in disease incidence.

Future strategies: Additional sources of resistance to TSWV in groundnut cultivars are necessary for cultivation in diverse agro-ecosystems. Both conventional breeding and non-conventional approaches using genetic engineering methods are being attempted to incorporate resistance to TSWV in groundnut. Spanish-type groundnut lines transformed with TSWV nucleocapsid protein (NP) gene had higher incidence of spotted wilt than the non-transformed control. But peanut cultivar Marc 1 transformed with NP gene of TSWV had lower spotted wilt incidence comparable to moderately resistant cultivar Georgia Green. Yang *et al.* (2004) have evaluated transgenic peanut (Marc 1) progenies that contained the NP gene of TSWV under field conditions at different locations in Southern USA. They observed significant lower incidence of spotted wilt in the transgenic peanut progeny as compared to the non-transgenic checks. Further, they suggested that this transgenic event could be used in a traditional breeding programme to enhance host resistance. Cultivar AT 120 transgenic for the antisense nucleocapsid gene of TSWV also had lower incidence of spotted wilt (reviewed by Cullbreath *et al.*, 2003). The research on conventional and non-conventional approaches needs continuation to identify and/or to develop promising groundnut cultivars. Recently, Schwach *et al.* (2004) have transformed the tobacco with gene construct comprising NP gene of TSWV and 5′ non-translated leader sequence of *Plum pox virus* as a translation enhancer. Such transformed tobacco plants

conferred resistance to TSWV and GRSV probably by blocking systemic spread. It is worth to extend this approach to groundnut.

3. *GROUNDNUT BUD NECROSIS VIRUS* (GROUNDNUT BUD NECROSIS)

Bud necrosis of groundnut was first reported from India (Reddy *et al.*, 1968). Later this disease was reported from South and Southeast Asia including China, under several different names viz. bud blight, groundnut mosaic, groundnut ring mosaic, bunchy top, chlorosis, ring mottle, ring spot and spotted wilt from India (Reddy, 1988). The causal virus of this disease was initially identified as TSWV in India (Ghanekar *et al.*, 1979). After virus purification and characterization, the virus involved in bud necrosis was identified as a distinct tospovirus and named as GBNV, which is placed in Serogroup IV of the genus *Tospovirus* (Reddy *et al.*, 1992; Adam *et al.*, 1993; Satyanarayana *et al.*, 1996a). It also infects several other crops (mungbean, urdbean, cowpea, soybean, sunflower, carrot, tomato, potato, cotton, *Lablab purpureus*) (Bhat *et al.*, 2001; Bhat *et al.*, 2002a; Thien *et al.*, 2003; Jain, 2004; Jain *et al*., 2004). GBNV has become increasingly important in the production of groundnut in South and Southeast Asia. Its incidence in groundnut in India is variable from field to field and from area to area even in a given season (from <1% to >70%). Crop losses worth up to US$89 million from India were reported (Reddy and Devi, 2003).

Basic properties and ecology of GBNV are very similar to TSWV. GBNV is mechanically transmissible and also transmitted by thrips vector, *Thrips palmi* in a persistant manner (Reddy and Devi, 2003). Thrips can acquire the virus only in their larval stage but both nymphs and adults can transmit the virus. It is not seed transmitted in groundnut or other species that it infects. It has extensive host range covering cultivated crops, ornamentals and weeds (Reddy and Wightman, 1988). The virus has been successfully purified (Satyanarayana *et al.*, 1996a). The virions are enveloped spherical or pleomorphic measuring 80-120 nm in diameter. Each virion contains three nucleocapsids, each containing genomic RNA tightly associated with nucleoprotein (N) of 31 kDa. The envelope contains surface glycoproteins (G1, G2). The genome is ambisense, tripartite, psuedocircular ssRNA. Size of the genome segments is 8911 for L RNA, 4801 for M RNA and 3057 for S RNA (Satyanarayana *et al.*, 1996a; Gowda *et al.*, 1998). The genomic organization and expression strategies of GBNV and TSWV are similar.

3.1 Symptoms

The symptoms of bud necrosis caused by GBNV, and spotted wilt caused by TSWV are similar on groundnut. Symptoms first appear in

young leaflets as chlorotic spots or mottling that may develop into chlorotic and/or necrotic rings and streaks. When temperatures are above 30°C, the petioles bearing fully expanded leaves with such initial symptoms usually become flaccid and droop. The necrosis from such leaves soon spreads to terminal growing buds. If the plant is young, it may become totally necrotic. Early infection causes stunting and sometimes proliferation of axillary shoots (Fig. 1 A). Leaflets on proliferated axillary shoots are small in size and exhibit puckering, chlorosis, mosaic, oak leaf line patterns and distortion of lamina. Entire plant shows bushy appearance and often confused with infection by other viruses like peanut stunt and groundnut chlorotic rosette. Such highly stunted bushy plants are usually difficult to notice in the fields as they often covered by the canopy of the adjacent healthy plants. The roots become dark, possess few nodules and are stunted. The early-infected plants never produce any worthwhile pods. Any seed produced on early-infected plants are small, shriveled and testae have red, brown or purple mottling. Late infected plants may produce seed of normal size but often testae mottled (Reddy, 1991).

3.2 Molecular diagnosis

In simple laboratories biological assays are useful for initial tentative diagnosis of the virus. This can be carried by sap inoculating the extracts from suspected plant samples to selected diagnostic hosts like *Vigna unguiculata* (local, concentric, chlorotic or necrotic rings), *Petunia hybrida* (necrotic local lesions), *Vinca rosea* (black tar like local lesions) and *Nicotiana glutinosa* (local necrotic lesions) (Reddy, 1991) the stability characteristics of the virus [longevity *in vitro* (LIV) and Thermal inactivation point (TIP)] can be used in conjunction with other methods to identify the virus. GBNV is unique in that it is transmitted only by *T. palmi*.

Good quality polyclonal antibodies to the purified virus or recombinant nucleocapsid protein have been produced at ICRISAT. Initially haemagglutination technique has been optimized for its detection (Rajeswari *et al.,* 1981). Later the widely used ELISA formats have been used for routine detection of the virus in plant samples (Hobbs *et al.,* 1987) and to determine its antigenic relationships with other tospoviruses (Reddy *et al.,* 1992). RT-PCR has been applied for sensitive detection of GBNV infections. Immunocapture-PCR allows reliable amplification of target virus sequences from peanut and other crops (Thien *et al.,* 2003; Jain *et al*., 2004). Primers derived from the nucleocapsid gene are able to amplify the target sequences in a specific and reproducible manner. This technique is also used to distinguish the tospoviral infections.

3.3 Phylogenetic relationships

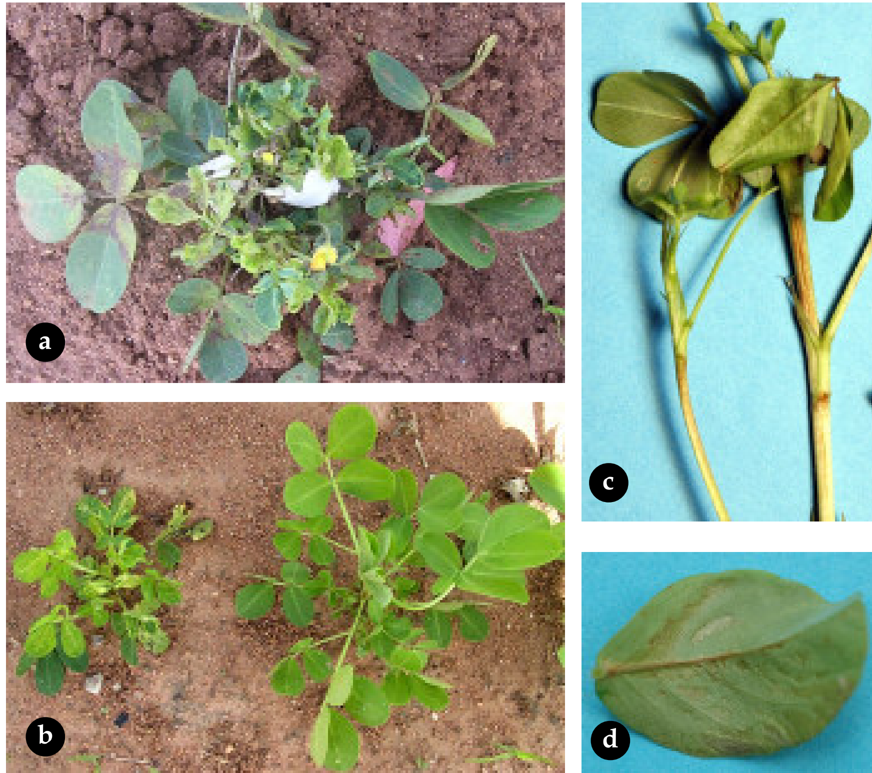

Fig. 1 (a) *Groundnut bud necrosis virus* infected groundnut; (b) *Indian peanut clump virus* infected groundnut (left), and healthy plant (right) c, d. Symptoms of *Tobacco streak virus* on groundnut. Note brown necrotic streaks on stems (c), and irregular etching on leaf lamina and necrotic symptoms on mid-rib (d).

Host range properties, thrips transmission, virion morphology and antigenic relationships of the virus have been initially used to identify and classify tospoviral infections including GBNV (Sreenivasulu *et al.*, 1991a; Reddy *et al.*, 1992; Adam *et al.*, 1993). This approach of classifying different tospoviruses into distinct species and serogroups is sometimes confusing and misleading. Therefore, comparison of their genome sequence similarities was used for reliable identification and classification. From 1990 onwards the genomes of several tospoviruses were either partially or completely sequenced and their phylogenetic relationships were determined (Moyer, 1999). The L, M and S RNAs of GBNV have been completely sequenced and its phylogenetic relationships have been determined (Satyanarayana *et al.*, 1996a; Gowda *et al.*, 1998). GBNV is placed in serogroup IV based on N or NSs protein sequences. It is serologically distinct from the members of other serogroups I, II, III and V. The N gene

sequences of several natural isolates from different crops were compared with the N gene of type GBNV (Bhat *et al.,* 2002a; Thien *et al.,* 2003; Jain *et al*., 2004). The comparative sequence analysis revealed that the tospovirus isolates from different crops in India shared more than 95% sequence identity and thus they have been identified as strains/isolates of GBNV. Recently, Akram *et al.* (2004) analyzed the nucleotide and amino acid sequences of the movement protein (NSm) genes of five isolates of GBNV originating from different hosts and regions of India (cowpea and tomato from Kerala, groundnut from Tamil Nadu and potato from Madhya Pradesh and Rajastan). They shared 98-100 % identity at amino acid levels with type GBNV suggesting that they have a common origin and adapted to different plant species. Tospoviruses infecting grain legumes around Delhi have been identified by serology and nucleic acid hybridization as strains of GBNV (Bhat *et al.,* 2001; Bhat *et al.,* 2002a).

GBNV shares 82% sequence similarity in N gene with another tospovirus, *Watermelon bud necrosis virus* (WBNV) reported from India (Jain *et al.,* 1998). Although WBNV can be transmitted to groundnut by artificial inoculations, but there are no reports of its natural occurrence on groundnut. GYSV, a distinct tospovirus that belongs to serogroup V of tospoviruses occurs widely on groundnut in India and Thailand. Both the viruses differ in symptomatology on groundnut and experimental hosts, thrips vector transmission, serology and nucleotide sequence of genome (Reddy *et al.,* 1991; Satyanarayana *et al.,* 1996b; 1998).

3.4 Management approaches

The primary sources of virus inoculum include a range of hosts (crops, ornamentals, weeds), which can sustain virus infection and support thrips vector multiplication (Reddy *et al.,* 1983a). The incidence and progress of the disease is dependent on several environmental and cropping practices which influence multiplication and spread of thrips. *Ageratum conyzoides* has been shown to support GBNV and vector multiplication. Primary spread is by thrips coming from other crops or weeds. Secondary spread from infected groundnut plants within a field was not established conclusively. Disease incidence continues to depend on infection by viruliferous thrips that acquire the virus from alternative hosts and thus bud necrosis disease of groundnut is mostly monocyclic type.

Management of bud necrosis disease depends upon the control of thrips vectors. Even though several weeds have been identified as sources of both virus and vectors, their eradication in the tropics is not practical. Insecticidal control of thrips was not effective in reducing the incidence. Cultural practices found to be more promising in reducing the disease incidence (Reddy, 1998). Depending on the arrival of principal thrips vectors

the sowing dates in the growing season in endemic areas need to be adjusted so that the good crop canopy can develop before infection occurs and thus disease incidence can be reduced. Intercropping of groundnuts with quick growing cereals like sorghum, pearlmillet or maize can reduce the disease incidence by acting as a barrier for thrips colonization. Rogueing of early-infected plants in the fields can create gaps, which can lead to increased incidence.

Work at ICRISAT has identified some groundnut cultivars and breeding lines that have field resistance to GBNV. They are ICGV 86029, 86031, 86388, 91239, 91245, 91246 and 91249. GBNV resistant groundnut cultivars ICGS 44, ICGS 11 were released in India. In Thailand, two peanut lines (ICGV 86388 and IC 10) were found to be resistant to GBNV (Pensuk *et al.,* 2004). Further, they analysed the field performance of populations derived from three crosses involving above two resistant peanut lines and one susceptible (KK 60-1) line. Their observations indicate that multiple genes control the bud necrosis disease resistance trait. The two resistant peanut lines probably differ in some of these resistance genes. At ICRISAT, groundnut cultivar JL 24 was transformed with nucleoprotein gene of GBNV and T2 transgenic events are being evaluated for virus resistance (K. K. Sharma, Personal Communication). If successful, these events will offer reliable resistance to GBNV that can also be bread into other groundnut cultivars through back cross breeding programs.

4. *TOBACCO STREAK VIRUS* (GROUNDNUT STEM NECROSIS)

Natural occurrence of TSV on groundnut was reported from India, Pakistan, South Africa and Brazil, but it has become economically important recently in Southern India. It was first recognized in 2000, infecting over 225,000 hectares of groundnut in Andhra Pradesh, India, and resulted in yield loss exceeding US $ 65 million. TSV has a wide natural host range, and is economically important in several crops like sunflower, cotton, soybeans, tobacco, cowpea, mungbean, sun-hemp, greengram, blackgram and bhendi (okra) (Bhat *et al.,* 2002c; Prasada Rao *et al.,* 2003[a]; Babu *et al.,* 2003). In sunflower it is considered to be a major yield-limiting factor in India (Winter and Ravi, 2003). Certain identified alternate crops in Andhra Pradesh are sunflower, safflower, cotton, cowpea, urdbean and mungbean; flowering ornamentals like marigold and weeds like *Acalypha indica, Abutilon indicum, Achyranthes aspera, Calotropis gigantea, Cleome viscosa, Commenlina benghalensis, Croton sparsiflorus, Digera arvensis, Euphorbia hirta, Euphorbia geniculata, Lagasca mallis, Leucas aspera, Parthenium hysterophorus* and *Tridax procumbens* (Prasad Rao *et al.,* 2003).

TSV is a type species in the genus *Ilarvirus*, family *Bromoviridae* (Van Regenmortel *et al.*, 2000). It is readily sap transmissible and naturally transmitted through pollen from infected plants with the aid of thrips, such as *Scirtothrips dorsalis, Frankliniella schultzeii, F. fusca, Thrips palmi* and *Megalurothrips usitatus* (Reddy *et al*., 2002; Prasada Rao *et al.,* 2003[a]). It is seed transmitted in soybean, but limited tests in groundnut and sunflower indicated that it is not seed-borne in these crops. The virus has wide experimental host range. Several plant species produce either chlorotic, necrotic local or systemic symptoms. TSV from groundnut was purified and the virions are quasi-isometric or occasionally bacilliform measuring 25-35 nm in diameter. The M_r of coat protein is 28 kDa. In addition, the purified virus preparation also contained a minor polypeptide of 57 kDa, probably a dimer of 28 kDa. The genome of TSV consists of positive sense, linear, tripartite ssRNAs with 5′ terminal cap structures. The 3′ terminus is not polyadenylated, sometimes form strong t-RNA like secondary structure. The total genome is approximately 8 kb. The three RNA species of TSV that infect other plant species have been sequenced. The coat protein sequence of several Indian isolates is available and they share more than 95% sequence similarity (GenBank accession numbers AY940153, AY606074, AY510128, AY510127, AY510126).

4.1 Symptoms

Initial symptoms on groundnut appear a week after infection. Young leaflets exhibit large necrotic lesions, which subsequently coalesce and cover the entire lamina. Later this leads to the total necrosis of the leaflets and spreads to the petiole, stems and growing buds (Fig. 1 C, D). Necrotic spots also occur on the shells of the pods. In severe infection the entire plant becomes necrotic and dies prematurely. In case of late infection, symptoms are confined to only terminal leaflets on a single branch, which may show necrosis. TSV symptoms in groundnut are often confused with those caused by GBNV. Necrosis on stems and petioles and necrotic spots on shells are characteristic of TSV infection. Whereas GBNV induces chlorotic ring spots on leaves and cause proliferation of axillary shoots.

4.2 Molecular diagnosis

Field diagnosis based on visual symptoms in groundnut may often mislead as the symptoms of this virus infection are confused with the symptoms of GBNV. Due to this reason the natural occurrence of TSV infections is probably unnoticed in farmers' fields. However, careful examination of the diseased groundnut plants will reveal the possible infection with either TSV or GBNV. Both the viruses induce necrosis of terminal leaflets. TSV often induces total stem necrosis and necrotic lesions on pods. GBNV often causes axillary shoot proliferation with distorted

leaflets and this has not been observed in TSV infections. For distinguishing the causal viruses of groundnut bud necrosis and stem necrosis, bioassays on selected diagnostic hosts (*Vigna unguiculata, Phaseolus vulgaris* cv Topcrop) are useful in initial tentative disease diagnosis (Prasada Rao *et al.,* 2003[b]). The virus isolate from groundnut has been purified and a good quality high titred polyclonal antiserum produced at ICRISAT and used for detection by ELISA (Reddy *et al.,* 2001; Prasada Rao *et al.,* 2003[b]). TSV can be detected by nucleic acid hybridization technique in both leaf and pollen extracts. The partial genome sequence (RNA 3) of the virus has facilitated to design the primers for PCR amplification of coat protein gene. RT-PCR is used in the detection of the virus and in identification of TSV infections in several crop plants in India (Reddy *et al.,* 2002; Bhat *et al.,* 2002b).

4.3 Phylogenetic relationships

The groundnut virus isolate was initially identified based on host range, virus stability, virion morphology, antigenic relationships with TSV and partial genome sequence data (Reddy *et al.*, 2002). TSV isolate from groundnut positively reacted with TSV-WC antiserum. The nucleotide sequence of RNA 3 shared 88.4% similarity with TSV-WC sequences available in the databases and thus it was considered as a strain of TSV. Subsequently, several isolates from naturally infected crops in India were isolated, their genomes were partially sequenced and identified them as strains of TSV (Arun Kumar *et al.,* 2004).

4.4 Management approaches

Diseased plants in the farmers' fields are found to be usually high near the field bunds or waste lands with weeds. Several other crops and weeds have been identified as sources of virus and thrips. Transmission can only occur through infected pollen with the aid of several thrips species (Prasada Rao *et al*., 2003). Groundnut seed does not carry infectious virus to initiate primary infection cycle. Parthenium, a ubiquitous weed, harbors both the virus and the thrips that visit its flowers carry the virus. TSV infected groundnut plants may not serve as a source for secondary spread in the same field or for primary spread to the near by fields because pollen from groundnut flowers is not an efficient source of inoculum. TSV incidence in groundnut fields was correlated with the infected parthenium near the field bunds (Prasada Rao *et al.,* 2003[b]). Removal of the weeds including parthenium from the vicinity of the groundnut fields is expected to reduce the disease incidence in the fields. Border and inter-cropping with maize, pearlmillet or sorghum around the groundnut fields may decrease the disease incidence by obstructing thrips movement and reduces the TSV incidence. Rogueing of early-infected plants may not limit or restrict further

spread of the disease. Cultivation of groundnut near sunflower and marigold should be discouraged because they act as a source of virus and/or thrips. Maintenance of sufficient plant density is important to discourage landing of thrips. Seed treatment with systemic insecticides (imidacloprid) may prevent vector infestation at early stages of crop growth. Limited germplasm screening revealed that groundnut varieties ICGV# 92267, 99029, 01276 and ICG 94379 show consistently low disease incidence. *Arachis chacoense* (ICG 4983) infected with TSV with no symptoms. Efforts are underway to develop transgenic resistance to TSV by exploiting its coat protein gene (K. K. Sharma, Personal Communication).

5. *GROUNDNUT ROSETTE AND GROUNDNUT ROSETTE ASSISTOR VIRUSES* (GROUNDNUT ROSETTE)

Groundnut rosette disease is by far the most destructive disease in Sub-Saharan Africa (SSA). It was first reported in 1907 from Tanzania (reviewed by Naidu *et al.,* 1999a). Since then it has been reported in several other Sub-Saharan African countries, Malawi, Nigeria, Uganda, Senegal, Burkina Faso, Cote d'Ivory, South Africa, Niger and also from Kenya (Wangai *et al.,* 2001). Symptoms similar to this disease have been reported in some countries of Asia and South Africa, but they have not been unequivocally confirmed. Therefore this disease is considered to be endemic to groundnut growing countries of sub-saharan Africa and Madagascar. Epidemics of groundnut rosette disease in Sub-Saharan Africa often significantly reduced the groundnut production. For example, in 1975 an epidemic in northern Nigeria destroyed nearly 0.7 million hectares of groundnut, estimated loss of US$250 million. Recurrent epidemics have limited production to below the pre-1975 yields. Similarly in 1995 epidemic occurred in eastern Zambia affected approximately 40,000 hectares, resulting in losses up to US$ 4.89 million. In Malawi, during 1994-95 epidemics lead to reduction in area of peanut production by 23% (Naidu *et al.,* 1999a; Taliansky *et al.,* 2000). Yield losses due to this disease depend on the growth stage of the plant when infection occurs. So if infection occurs before flowering, over 90% loss in pod yield due to either chlorotic or green rosette disease may result. Yield loss is variable if infection occurs between flowering and pod maturing stage, whereas subsequent infections cause negligible effects.

The etiology of the rosette is complex involving three agents, *Groundnut rosette assistor virus* (GRAV) of the genus *Luteovirus*; *Groundnut rosette virus* (GRV) of the genus *Umbravirus* and Satellite RNA (Reddy *et al.,* 1985a; 1985b; Naidu *et al.,* 1999; Taliansky *et al.,* 2000). These three components are intricately dependent on each other, and all three play crucial role in biology and perpetuation of the disease. Unlike the other members of

Luteoviridae family, which cause often yellowing, reddening and /or stunting of their host plants, GRAV infection alone is asymptomatic in groundnut. GRAV has been purified and characterized. It has 25 nm diameter non-enveloped, isometric particles that are mostly confined to the phloem tissues. GRAV is transmitted by aphid species (*Aphis craccivora*) in a circulative non-propagative manner, but not through seed or by sap inoculation. Its genome is positive sense, linear, unipartite, ssRNA (about 6100 nts). GRAV assists the transmission of umbravirus, GRV, in the rosette complex. It is serologically related to several *Polerovirus* species (Rajeswari *et al.,* 1987).

GRV has no definite particle morphology and they are devoid of coat protein. It depends on a helper virus from *Luteoviridae* for aphid transmission. GRV is sap transmitted, but not through seed. It is aphid transmitted in a persistent manner in the presence of helper virus (GRAV) and sat-RNA. GRV alone causes asymptomatic infections. GRV genome is positive sense, linear, unipartite ssRNA (4019 nts). In tissues infected with GRV alone, no typical nucleoprotein particles have been observed by electron microscopy. GRV infected tissues contain two subgenomic RNAs corresponding to ORF 3 and 4 besides full length genomic RNA. It is autonomous in its replication, but presence of sat-RNA in the source plant is essential for GRAV-assisted aphid transmission. Hence all the three components must occur together for functional transmission by aphid vectors and subsequent disease development (Naidu *et al.*, 1999b).

The GRV satellite RNAs associated with chlorotic and green rosette disease in different regions of Africa are 895-903 nucleotides long and are atleast 87% identical. The sat RNA contains up to 5 ORFs in either positive or negative sense, but the role of any proteins expressed from these ORFs is unknown. Evidence suggests that sat RNA plays a key role in symptom expression. A few sat RNA variants are known to induce only mild symptoms in *Nicotiana benthamiana* and drastically reduced the replication of GRV (Block *et al.,* 1994; Taliansky *et al.,* 2000). GRV and sat-RNAs are not phloem limited and both are transmitted through sap inoculations.

5.1 Symptoms

Groundnut is the only known natural host of the rosette disease complex. Plants that are infected under laboratory conditions are listed by Taliansky *et al.* (2000). The three major forms of the disease reported are chlorotic rosette, green rosette and mosaic rosette. The chlorotic rosette is ubiquitous in sub-saharan Africa, while green rosette is distributed in West Africa, Uganda and Angola. Mosaic rosette was recorded only in east and central Africa. Considerable variation has been reported within chlorotic rosette (Naidu *et al.,* 1999a). Faint chlorotic mottling first appear on young leaflets and subsequent leaflets show bright yellow color

interspersed with green islands. The early-infected plants are severely stunted due to shortening of internodes. A few pods that are formed may not contain marketable seeds. Another form of chlorotic rosette is green mottle, which is characterized by mild chlorotic mottling with isolated flecks. Older leaflets are small and dark green and exhibit outward rolling. If the older plants are infected, the symptoms may be confined to apical branches.

Green rosette resembles symptoms of peanut clump caused by PCV (Reddy *et al.,* 1999), making it difficult to differentiate and determine the distribution and impact of green rosette disease on groundnut production. Young leaflets show mild mottling and isolated flecks. Older leaflets are reduced in size, exhibit downward rolling and are not distorted. Diseased peanut plants are severely stunted and are darker green than healthy plants. In mosaic rosette young leaflets show conspicuous mosaic symptoms. Later symptoms resemble those of chlorotic rosette. Stunting is less pronounced as compared to chlorotic rosette. Variability in disease symptoms could be due to diversity among the causal agents, differences in genotype response, variable climatic conditions and mixed infections with other viruses.

5.2 Molecular diagnosis

Groundnut rosette disease can be tentatively diagnosed in the farmers' fields based on the characteristic symptoms in groundnut or by sap inoculation onto *Chenopodium amaranticolor*. Development of symptoms on this host indicates the presence of GRV. But this bioassay is not reliable as the plants are subjected to widely fluctuating temperatures of SSA. Groundnut rosette disease complex is readily transmitted by *Aphis craccivora* and hence can be distinguished from fungus transmitted 'clump' disease. Immuno-specific electron microscopy is used to detect GRAV (Rajeswari and Murant, 1988). Improved diagnostic methods include triple antibody sandwich-ELISA for the detection of GRAV, dot-blot hybridization assay for the detection of GRV and sat-RNA (Rajeswari *et al.,* 1987; Blok *et al.,* 1995). The presence of sat-RNA in disease complex was initially revealed by agarose gel electrophoretic analysis of total RNA extracts from the diseased leaves (Breyel *et al.,* 1988) and this approach has been effectively used as a diagnostic tool (Olorunju *et al.,* 1991; 1992). RT-PCR has been used for concurrent detection of all the three groundnut rosette disease agents in plants and aphids (Naidu *et al.,* 1998).

5.3 Phylogenetic relationships

The complete nucleotide sequence (4019 nts long) and genome organisation (4 ORFs) of GRV are known (Taliansky *et al.,* 1996). The

sequences of 10 variants of sat-RNA (895-903 nts) associated with chlorotic and green rosette diseases from different regions of Africa have been reported, and they are atleast 87% identical (Blok *et al.,* 1994). Sequence diversity was studied in the CP gene of GRAV, ORF 3 and 4 of GRV and sat-RNA of GRV obtained from field isolates from Malawi, Nigeria and Kenya (Deom *et al.,* 2000; Wangai *et al.,* 2001). Sequence analysis showed that GRAV CP gene was highly conserved (97-99%) independent of its geographic source. The nucleotide sequence of the overlapping ORFs 3 and 4 of GRV was also highly conserved (98-100 %) from isolates within a geographical region, but less conserved (88-89 %) between isolates from the two distinct geographical regions (Malawi and Nigeria). The GRV sequences of Kenyan isolate were close to the Malawian (95-96%) than to the Nigerian (87-88%) isolates. Phylogenetic analysis of the ORFs 3 and 4 of GRV isolates revealed that Malawian GRV isolates are distinct from Nigerian GRV isolates (Deom *et al.,* 2000). Similarity within the satRNA sequences ranged from 88- 99%. Phylogenetical analysis of satRNA sequences showed clustering within the satRNA isolates according to country of origin, as well as within isolates from two distinct regions of Malawi. The sequences of satRNA from Kenya shared 89-94% identity with those from Malawi and Nigeria. A closer sequence relationship was reported between Kenyan and Malawian isolates (Wangai *et al.,* 2001).

5.4 Management approaches

The groundnut rosette disease epidemiology is complex as it involves interactions between two distinct viruses and a satRNA, an aphid vector and the host plant in the unpredictable environments of SSA. None of the three agents of rosette complex are seed-borne, and therefore primary infection must be introduced into the crop by viruliferous aphids. The possible sources from which the disease could spread are infected groundnut plants surviving from cropping seasons. The source of viruliferous aphids that initiate groundnut rosette disease is unknown. As the disease is endemic to SSA and its offshore islands, it is presumed that there are alternate plants from which the virus spreads into groundnut with the help of polyphagous *A. craccivora.* Although host plants other than groundnut for GRAV and/or GRV and satRNA have been identified under experimental conditions (Hull and Adams, 1968; Reddy *et al.,* 1985a; 1985b), but under natural conditions these agents were detected only in groundnut in SSA. The spread of groundnut rosette is complicated because a single aphid may not always transmit the three agents. Plants that show symptoms but lack GRAV play no role in the spread of the disease because the coat protein of GRAV is required for encapsidation and transmission of GRV and satRNA. Therefore the number of plants that possess all the three agents play a crucial role in the secondary spread of the disease in

a given field, while the total number of plants showing symptoms irrespective of having GRAV influences yield.

Several methods have been investigated to mange rosette disease. They include pesticides to reduce vector populations, cropping practices to delay onset and spread of both vector and disease, and cultural practices, but only limited success has been achieved with each of these approaches (reviewed in Naidu *et al.,* 1999a).

Sources of resistance to groundnut rosette disease were first identified in groundnut landraces of late maturing Virginia type in West Africa. Resistance to this disease is also identified in the early maturing Spanish type. Resistance identified in races of Virginia type was used in breeding programmes throughout SSA and has contributed to the development of several resistant cultivars (eg., RMP 12, RMP 91, KH 241-D and RG 1) (Naidu *et al.,* 1999a). Resistance among these cultivars was found to be effective against both chlorotic and green rosette and was governed by two independent recessive genes (Nigam and Bock, 1990), but these cultivars were not popularly adopted due to their long duration nature. A subsequent research combined groundnut rosette disease resistance with early maturing (92-110 days) high yielding Spanish types suitable for diverse ecosystems of SSA. This approach resulted in identification of several genotypes with good levels of resistance to chlorotic rosette disease in various maturity groups (Bock *et al.,* 1990; Subrahmanyam *et al.,* 1998; Naidu *et al.,* 1999a). It is interesting to note that none of the rosette resistant groundnut cultivars and germplasm lines identified so far has resistance to GRAV (Olorunju *et al.*, 1991; 1992; Subramanyam *et al.,* 1998). In the resistant cultivars and germplasm lines, resistance was to GRV, which results in indirect resistance to satRNA and therefore such genotypes do not develop symptoms (Bock *et al.,* 1990). But resistance to GRV does not amount immunity and can be overcome under high inoculum pressure and/or adverse environmental conditions. Most of the earlier studies on inheritance of disease resistance were based on visual symptoms and are applicable only to GRV and its sat-RNA, but not to GRAV. Immunity to GRAV was identified in several wild *Arachis* species or accessions (Murant *et al.,* 1991). Subramanyam *et al.* (2001) have identified several wild *Arachis* species resistant to all the three causative components of groundnut rosette. Conventional breeding and/or biotechnological approaches can be used to transfer immunity to GRAV into cultivated groundnut. Resistance to the aphid vector has been identified in groundnut genotype EC 36892 and this can be exploited in the resistance breeding programs (Padgham *et al.,* 1990). Attempts have been made to exploit pathogen-derived resistance (GRAV replicase and CP genes, movement protein genes and/or satRNA derived sequences) to groundnut rosette disease to develop broad-based agronomically superior groundnut cultivars (Taliansky *et al.,* 1998). At

ICRISAT transgenic groundnut carrying GRAV coat protein gene was developed, and these events will be evaluated in South Africa for rosette resistance (K. K. Sharma, ICRISAT, Personal Communication).

6. *PEANUT CLUMP VIRUS* (PEANUT CLUMP)

Clump disease was first reported in 1927 in groundnut crop in India and in 1931 a similar disease in groundnut was reported from West Africa (reviewed by Reddy *et al.,* 1999). Clump disease was reported from Asian countries: India (from Andhra Pradesh, Gujarat, Punjab, Rajastan and Tamil Nadu states) and Pakistan (Sindh and Punjab states); and West African countries: Burkina Faso, Niger, Mali, Cote d'Ivory, Gambia and Senegal (Reddy *et al.,* 1999). They are transmitted through seed and by soil-borne plasmodiophoromycete fungi, *Polymyxa graminis* and by mechanical sap inoculation (Reddy *et al*., 2005). The viruses survive for long periods in resting spores of the fungus and contribute to the perpetuation of the disease. The economic importance of this disease was recognized following systematic surveys in the Indian subcontinent as well as in parts of West Africa. The clump virus that occurs in Indian sub-continent is referred as *Indian peanut clump virus* (IPCV) where as the virus that occurs in Africa is called as *Peanut clump virus* (PCV). Both PCV and IPCV are important because they not only infect groundnut but also several other economically important crops like sugarcane, few cereals and millets and pigeonpea. In groundnut almost 100% crop loss results when the disease occurs in the early growing season, and up to 60% yield loss in late infected plants (Reddy, 1991). The annual loss due to this disease on global scale has been estimated to exceed US$38 million (Reddy and Devi, 2003). Further, clump disease also has quarantine importance, as the causal viruses are transmissible through groundnut seed, and also thorough seed of pearl millet, finger millet, foxtail millet, wheat and maize (Delfosse *et al.,* 1999).

PCV and IPCV are distinct species in the genus *Pecluvirus* (Van Regenmortel *et al.,* 2000). IPCV particles are bipartite rod shaped, 249×24 and 184×24 nm. PCV particles contain two predominate lengths, 245×24 and 190×24 nm. Both viruses contain single capsid protein of 24 kDa. The genomes of both the viruses are positive sense, linear, bipartite, ssRNA and sequenced. The length of IPCV RNA 1 is 5841 nts, and RNA 2 is 4507 nts (Miller *et al.,* 1996; Naidu *et al.,* 2003). Whereas the length of PCV RNA 1 is 5897 nts and RNA 2 is 4504 nts (Manohar *et al.,* 1993; Herzog *et al*., 1994; Naidu *et al.,* 2003). Both the viruses have similar genome organisation. RNA 1 contains three ORFs which encode proteins of size 131, 119 and 15 kDa. The 5′ most ORF of RNA 1 encodes 131 kDa polypeptide, whereas 3′ ORF specifies 15 kDa polypeptide. The larger 190 kDa polypeptide is expressed as a read-through of the UGA termination codon of 5′ 131 ORF. The 190 kDa polypeptide contains domains with sequences similar to

proteins with methyl transferase, helicase and RNA-dependent RNA polymerase activities. No function inferred from the sequences of 15 kDa polypeptide. The RNA 2 contains 5 ORFs encoding 5 polypeptides. The ORF at the 5′end codes for the coat protein. The second ORF overlaps the first ORF by 2 nucleotides, and encodes a polypeptide of 39 kDa that is suspected to be involved in the transmission of the virus by fungal vector. Further downstream, separated by a 135 nucleotide intergenic region is triple gene block sequence that codes 51, 14, 17 kDa polypeptides. These are implicated in the movement of the virus from cell to cell. The 5′ non-coding region of RNA 1 is about 130 nts and of RNA 2 is between 390-500 nucleotides. There is no 5′ sequence feature to all pecluviruses. The 3′ nucleotides are about 300-350 in length and about 100 terminal nucleotides are almost identical among pecluvirus RNAs sequenced. This sequence similarity has facilitated the development of hybridization probe (3′ terminal 700 nts sequence of IPCV-H serotype), which can detect several IPCV serotypes as well as an isolate of PCV (Reddy *et al.,* 1999).

6.1 Symptoms

The symptoms induced by various IPCV isolates differed in several hosts (Nolt *et al.,* 1988). PCV isolates collected from Senegal, Burkina Faso and Niger induce varied symptoms in *Chenopodium amaranticolor.* However, symptoms incited by the IPCV and PCV in groundnut plants are similar. The symptoms of clump disease are often confused with green rosette. Clump disease affected plants usually occur in patches in the farmers' fields and they are highly stunted and appear green even at crop maturity stage. Hence the disease can be easily recognized in the fields. The disease recurs in the same areas year after year with marginal increase in the periphery of the patches in the successive groundnut crops. Earlier symptoms on young leaflets of groundnut are mottling, mosaic and chlorotic rings. When these leaflets mature, they turn dark green with or without faint mottling. The early infected plants become severely stunted (Fig. 1 B). Late infected plants may not show conspicuous symptoms but appear dark green with faint mottling on young leaflets. In late infected plants clumping is restricted to few branches. The diseased plants produce flowers and if the pods are formed, they are not well developed (Nolt *et al.,* 1988).

6.2 Molecular diagnosis

Diseased plants can be readily identified in the farmers' fields by its characteristic symptoms and patchy distribution of infected plants. *Chenopodium amaranticolor* is a good diagnostic host for both the viruses for confirmation by mechanical sap inoculation. The causal viruses have been purified and polyclonal antisera were produced. Both the viruses exist as serologically distinct and ELISA based tests have been used for the

detection of these viruses (Huguenot *et al.,* 1989; Manohar *et al.,* 1995). Nucleic acid hybridization using probe corresponding to 3′ terminal 700 nucleotides of IPCV-H was developed. It is known to detect IPCV serotypes as well as an isolate of PCV (Reddy *et al.,* 1985c). The conserved nucleotide sequence at the 3′ end that is identical in both the viruses facilitated the development of hybridization probe, which can detect several IPCV serotypes as well as an isolate of PCV (Reddy *et al.,* 1999). As the genome sequence information is available, it is easy to design primers essential for their detection by RT-PCR.

6.3 Phylogenetic relationships

IPCV and PCV exist as serologically distinct isolates. IPCV isolates have been grouped into three distinct serotypes viz. IPCV-H (Hyderabad), IPCV-D (Durgapura), IPCV-L (Ludhiana) (Nolt *et al.,* 1988). All IPCV isolates are found to be distinct from PCV isolates, and vice versa. A number of PCV isolates have been placed in 5 distinct species by using monoclonal antibodies. None of the seven monoclonal antibodies produced against PCV reacted with IPCV-D in TAS-ELISA (Huguenot *et al*., 1989; Manohar *et al.,* 1995). The sequence of RNA 1 of IPCV-H is similar to that of PCV and corresponding encoded polypeptides are 88%, 95% and 75% identical (Herzog *et al.,* 1994). The polypeptides encoded by pecluvirus RNA 1 show significant similarities with other rod-shaped viruses classified in *Furoviridae* (56% identity with polymerase of *Soil-borne wheat mosaic virus*). Recently, the complete nucleotide sequence of RNA 2 of four isolates of PCV and two isolates of IPCV were determined (Naidu *et al.,* 2003). Comparisons among the sequences of six isolates and two earlier published, revealed high degree of variability in size (between 4290 and 4652 nts) and nucleotide sequence identity (between 58% and 79%). The ORF4 that encodes the second of the triple gene block proteins is highly conserved (90-98% identical). Whereas ORF that encodes a protein with unknown function is less conserved (25-60% identical). The coat protein of the eight isolates showed amino acid identities between 37% and 89% and contained several conserved residues. The coat proteins also show significant similarity with the coat protein of *Barley stripe mosaic virus* (Genus *Hordeivirus*) (Wesley *et al*., 1994), and the triple gene block proteins resemble those of *Potato moptop virus* (Genus *Pomovirus*). Phylogenetic comparison, based on complete RNA 2 sequences, revealed that the eight isolates can fall into two distinct clusters with no geographical distinction between PCV and IPCV isolates (Naidu *et al*., 2003).

6.4 Management approaches

Both PCV and IPCV are soil-borne and transmitted by *P. graminis*. The vector in the soil, survives as highly resistant resting spores, is

responsible for the patchy appearance of the disease and annual recurrence. Eventhough the fungus can transmit the virus to dicot plants, it does not extensively colonize their roots and hence resting spores are seldom detected in such plants. Naturally virus infected groundnut plant roots fail to induce the disease when incorporated into sterile sand, whereas infected sorghum and pearlmillet roots could induce disease. For these reasons, dicot plants are considered as 'fortuitous' hosts that are unlikely to contribute to build-up of virus inoculum. On the other hand monocot crops like maize, pearlmillet and sorghum are regarded as 'preferred' hosts for *P. graminis* because of its high multiplication in them (Legreve *et al.,* 1999; Delfosse *et al.,* 2002). The virus is also transmitted through the seed of cereal crops. The clump disease often occurs in groundnut crops raised during the rainy season in India. During this season the ambient temperatures range from 25-40°C. The optimum temperature for infection by fungus vector is between 27° and 30°C. Below 23°C infection does not occur and fungus development is delayed, and this could be the reason for the absence of the clump disease in the crops raised during post-rainy season in India, during this season ambient temperatures range between 15-30°C. Rainfall also plays significant role in natural infection by *P. graminis* and IPCV. *P. graminis* is known to be favored by alternate watering and drainage and thus the disease commonly occurs in sandy soils. Continuous cropping with groundnut, cowpea and pigeonpea (fortuitous hosts) is likely to decrease the inoculum in the soil. On the other hand, intercropping with susceptible cereal crops can contribute to increase in incidence and spread of the disease. Hence, it was suggested that pecluviruses are not typical groundnut viruses, but actually graminaceous viruses that possibly co-evolved in tropical and sub-tropical areas with wild grasses and field crops such as millets and sorghum (Delfosse *et al.,* 2002).

Clump disease is difficult to control as it is a persistant soil-borne disease. However, its satisfactory control can be achieved by adapting cultural practices over a period of time. The studies on disease epidemiology in India have lead to formulation of the following cultural practices: (a) early sowing of groundnut crop before the onset of monsoon rains; (b) use of pearlmillet as bait crop to reduce the inoculum load in the soil. To achieve this, bait crops are advised to plant soon after the onset of monsoon preferably under irrigation and up rooted in three weeks after germination. (c) avoiding rotation with highly susceptible cereal crops such as maize and wheat; (d) sowing peanut during post rainy season; (e) continuous cropping with dicot hosts to reduce the inoculum in the soil and thus low incidence of disease and (f) soil solarization during hot summer months. This is achieved by covering well-irrigated soils with a transparent polythene sheet for atleast 3 weeks. Soil biocides are effective in reducing disease incidence but they are not economical to adopt. Further, the effective

biocides are known to be hazardous.

No resistance to IPCV was found in nearly 9000 *Arachis* germplasm lines screened. But resistance was identified in wild *Arachis* species, and is yet to be incorporated into cultivated peanut (Reddy and Devi, 2003). At ICRISAT groundnut cultivar JL24 was transformed with IPCV-H coat protein and replicase genes to induce pathogen-derived resistance. The transformed events are being evaluated in on-station trails (K. K. Sharma, Personal Communication).

7. *PEANUT STRIPE VIRUS* (PEANUT STRIPE)

Xu *et al.* (1983) first reported a virus from China resembling peanut stripe. Later Demski *et al.* (1984) reported a potyvirus isolated from peanut seed imported from China and named it '*Peanut stripe virus*' (PStV). This virus infection is endemic in Southeast Asia, China and in majority of groundnut growing countries, including India (McDonald *et al.,* 1998). Yield reductions by PStV in groundnut in Georgia were about 7% in experimental plots (Lynch *et al.,* 1988), but yield losses can reach up to 70% in early-infected plants. In northern China annual yield loss estimated at 200,000 tonnes of pods was reported (McDonald *et al.,* 1998). The seed-borne nature of the virus in groundnut is of considerable interest in the international exchange of groundnut germplasm, as this means of virus transmission can lead to the introduction of new strains of the virus into groundnut growing areas.

PStV naturally infects not only groundnut but also a few other crops like cowpea, soybean and sesamum and a few legume weeds, *Centrosema pubescens, C. macrocarpum, Calapogonium caeruleum, Crotalaria striata, Desmodium siliquosum* and *Peuraria phaseoloides* (Wongkaew, 1986; Sreenivasulu *et al.,* 1992; Demski *et al.,* 1993). PStV has moderate experimental host range (Demski *et al.,* 1984). It is sap transmissible and also through groundnut seed upto 37%. However, in naturally infected groundnut plants seed transmission rates are between 0-7%, generally less than 4%. Aphids like *Aphis craccivora, A. gossypii* and *Myzus persicae* transmit the virus in a non-circulative, non-persistant manner. The virus induces cytoplasmic cylindrical/pinwheel inclusions that are diagnostic to the family *Potyviridae.* The virus was purified and the virions are non-enveloped, flexuous filamentous particles measuring 752 x 12 nm. The coat protein of the virions consists of one polypeptide with M_r 33.5 kDa. The virus genome is a positive sense, linear, unipartite ssRNA and was completely sequenced (Flasinski *et al.,* 1996). HPLC peptide profiling of coat protein and genome sequence have revealed that it has more similarities to *Bean common mosaic virus* (BCMV), genus *Potyvirus* (McKern *et al*., 1989), and therefore suggested as a groundnut infecting strain of

BCMV. The PStV RNA genome has VPg (Viral protein genome linked) at its 5′ end and a poly (A) tail at its 3′ end. The genome contains a single long open reading frame (ORF) translated into a large polyprotein that is co- and/or post-translationally cleaved into final functional 8-10 proteins (Urcuqui-Inchima *et al*., 2001).

7.1 Symptoms

The virus initially causes stripes and green vein banding symptoms along lateral veins of groundnut leaflets. The virus isolates from different peanut growing regions of the world can induce diverse and distinct symptoms. The first recognized stripe isolate produces discontinuous stripes along the lateral veins on young leaflets of peanut. Older leaflets exhibit striping, mosaic in the form of green islands and an oak leaf pattern. The infected plants are slightly stunted (Demski *et al.,* 1984). Most other reported PStV isolates produce initially chlorotic flecks followed by mild mottle, blotch, or chlorotic ring mottle symptoms. An isolate that produces necrotic symptoms on peanut was also reported (Xu *et al.,* 1983; Chang *et al.,* 1990; Wongkaew and Dollet, 1990; Sreenivasulu *et al.,* 1992). The necrotic strain reported from Taiwan produces necrotic lesions on leaves, with the necrosis later extended to the mid-ribs, petioles and sometimes to the stems. This leads to stunting, severe mosaic and systemic foliar distortion or stripe symptoms (Chang *et al.,* 1990). A necrotic strain of the virus isolated from beggar weed and groundnut in USA causes a disease resembling TSWV (Sreenivasulu *et al.,* 1992).

7.2 Molecular diagnosis

Diagnosis of the disease at the field level caused by different isolates of PStV sometimes can mislead as they are known to induce varied symptoms in different groundnut cultivars in different countries. PStV produces chlorotic or necrotic local lesions on *Chenopodium amaranticolor*. It does not infect *Phaseolus vulgaris* cv Top crop and peas that are highly susceptible to peanut mottle, another commonly occurring potyvirus in groundnut. High quality polyclonal antiserum was produced to the purified virus and ELISA based tests have been successfully used to detect the virus in leaves as well as in groundnut seed. The antiserum of this virus also reacts strongly with blackeye cowpea mosaic, soybean mosaic, clover yellow vein mosaic and adzuki bean mosaic potyviruses, but not with peanut mottle virus. Monoclonal antibodies were also produced against this virus and used for the virus detection and in determining its antigenic relationships (Culver and Sherwood, 1988). Dot-blot hybridization using ^{32}P-labelled cDNA probes was used for the detection of the virus in peanut seeds (Bijaisoradat and Kuhn, 1988). Virus was readily detected by this test even in 1 mg of infected peanut seed tissue and judged as 8-10 times

more sensitive when compared to ELISA. RT-PCR has been shown to detect virus at picogram levels in groundnut seed (Dietzgen *et al.,* 2001). An immunocapture RT-PCR for the detection of PStV in seed lots was demonstrated to be more sensitive than ELISA (Gillaspie *et al.,* 2000). Multiplex PCR has been used for specific detection of four seed-borne groundnut infecting viruses viz. peanut stripe, peanut mottle, peanut stunt and cucumber mosaic viruses (Dietzgen *et al.,* 2001). They have also used duplex RT-PCR to differentiate the two potyviruses from the two cucumoviruses. The multiplex and duplex RT-PCRs could be useful in testing both groundnut plants and/or seeds for virus identification in epidemiological studies and seed testing on post-entry quarantine.

7.3 Phylogenetic relationships

PStV-like viruses causing stripe, blotch, green blotch, chlorotic rings, mild mottle or green mosaic in groundnut have been reported from different countries in Southeast Asia. All these isolates involved in these diseases are serologically indistinguishable from PStV. An adhoc committee on PStV nomenclature proposed that these virus isolates could be recognized as isolates of PStV to avoid further confusion (Demski *et al.,* 1988). However, the committee recommended that all the isolates should be tested under identical conditions to determine their exact relationships. To achieve this, Wongkaew and Dollet (1990) have collected 24 isolates of the virus from 8 groundnut-growing countries and compared them under identical conditions in France. They have placed 24 isolates based on reactions on specific hosts (groundnut, *Chenopodium amaranticolor,* cowpea) and antigenic relationships into eight strains viz. mild mottle, blotch, blotch-CP-N, stripe, blotch-stripe, chlorotic ring-mottle, chlorotic line pattern and necrotic. The HPLC profiles of coat protein tryptic digests from blotch, stripe and mild mottle strains of PStV have been compared. Such profiles are also compared with blackeye cowpea mosaic, clover yellow vein, bean yellow mosaic and soybean mosaic viruses (McKern *et al*., 1989). They have shown that the 3 strains of PStV have very similar peptide profiles and suggested them as strains of PStV. The peptide profiles of BlCMV also show the similarity with the profiles of PStV strains, indicating close structural relationships between these two viruses. Later it was suggested that PStV, BlCMV and SMV are the strains of BCMV (McKern *et al.,* 1989). PStV isolates from Thailand are considered to be strains of BCMV that can be distinguished from bean infecting strain of BCMV and BICMV through differences in the nucleotide sequence and host range (Higgins *et al.,* 1998). PStV strains have coat protein sequence variability below 10% and can be defined according to geographic origin and symptom type (Higgins *et al.,* 1999). The 3′ region (UTR, CP and part of NIb) of necrotic strain (1356 nts) of PStV was sequenced and found to have 92-95% homology to blotch strain

at the nucleotide sequence levels. Nucleotide sequence differences unique to the necrotic isolate were identified by comparison to the non-necrotic isolate of PStV (Pappu *et al.,* 1998). Nucleotide polymorphism in the coat protein gene sequences was utilized in designing oligonucleotides that were specific to the necrotic strains, and were employed in an assay to differentiate a necrotic strain from non-necrotic. The 3′ end mismatch in the oligonucleotides contributed to the differentiation of the strains. This approach facilitated rapid, sensitive and reliable detection and differentiation of PStV strains.

7.4 Management approaches

In nature PStV is transmitted in groundnut seed and by aphids, in a non-persistant manner. The primary cycle of the disease is initiated through seed-borne infection. In Thailand and USA certain weeds like *Centrosema pubescence, C. macrocarpum, Calopogonium caeruleum, Crotalaria striata, Desmodium siliquiosum Peuraria phaseoloides* and beggar weed have been reported as natural susceptible hosts. In the absence of seed-borne virus, primary cycle can be initiated by aphids that acquire the virus from weed hosts. Secondary spread in the peanut fields is mainly by aphid vectors. Since the primary source of virus inoculum is seed, planting should be done with seed lots obtained from disease-free areas. Attempts made to control PStV by using 10% milk suspension, metasystox or milk alternated with metasystox or pyrimidine carbamate (systemic aphicide) was ineffective (Demski *et al.,* 1993). Enforcing of strict quarantine regulations in countries where the virus is known to be restricted at certain locations is important to avoid introduction of the virus into the virus-free locations. Only certified groundnut seed is to be moved between the locations or countries. Application of plastic film mulch in groundnut fields in China is found to reduce PStV incidence.

Resistance to this virus could not be found in cultivated peanut. However, resistance to PStV has been identified in some wild *Arachis* species by sap inoculation (Culver *et al.,* 1987), but no attempts have been made to transfer this resistance to cultivated groundnut. Attempts to identify genotypes which do not transmit PStV through seed were unsuccessful. Pathogen-derived resistance has been deployed successfully to provide resistance to potyvirus infection in several crops (Dasgupta *et al.,* 2003). Genetically modified groundnut plants that carry viral coat protein gene exhibited high levels of resistance to PStV (Higgins *et al.,* 2004). But high level resistance or immunity can be induced in plants by triggering RNA silencing, an intrinsic defense mechanism against viruses (Waterhouse *et al.,* 2001). To trigger RNA silencing mechanism, transgenic groundnut plants that contained full length untranslatable form of CP gene and 3′ untranslated region of Indonesian blotch strain of PStV have

been produced. None of these regenerated plants expressed detectable amounts of PStV coat protein. However, transgene specific small RNAs, the products of RNA silencing, were detected in highly resistant lines but not in a susceptible transformed line. The transformed plants were highly resistant to PStV infection and the resistance was stably inherited over atleast five generations (Dietzgen *et al.,* 2004).

8. *PEANUT MOTTLE VIRUS* (PEANUT MOTTLE)

This virus was first reported from USA in 1961, and is present in all major groundnut-growing countries (Bock and Kuhn, 1975; Kuhn and Demski, 1984). It also naturally infects other crops like cowpea, French bean, soybean and white lupine (Brunt *et al.,* 1996). Demski and Reddy (1997) have considered this virus to be economically important globally. In Georgia losses due to this virus were estimated upto 20 – 70 % (Kuhn and Demski, 1975), and in India in susceptible groundnut cultivars crop losses may reach 40 %. Further, it has quarantine importance, as the virus is seed-transmitted. This virus has been identified as a distinct species in the genus *Potyvirus,* family *Potyviridae.*

Peanut mottle virus (PeMoV) is easily sap transmitted and transmitted through the groundnut seed to variable levels. This virus is also transmitted in a non-persistant manner by *Aphis craccivora, A. gossypii, Myzus persicae, Hyperomyzus lactucae, Rhopalosiphum padii and R. maidis* (Paguio and Kuhn, 1976; Highland *et al.,* 1981). When groundnut plants infected with PeMoV and PStV are used as a virus source plants, *A. craccivora* and *M. persicae* are able to transmit either PeMoV or PStV but not both viruses together (Sreenivasulu and Demski, 1988). The virus has a moderate host range. *Pisum sativum* is used as a virus propagation host and *Phaseolus vulgaris* cv Topcrop as an assay host. The virus induces pinwheel inclusions in the cytoplasm of infected tissues. The purified virions are non-enveloped, flexuous filamentous particles measuring 750 x 12 nm (Rajeswari *et al.,* 1983). The coat protein has M_r of 32 kDa. The genome of the virus is a positive sense, linear, unipartite, ssRNA and is completely sequenced (9500 nts) (Teycheney and Dietzgen, 1994). Several strains (mild mottle strains M1 and M2; necrosis N strain; sever mosaic S strain and chlorotic line pattern CLP strain) of PeMoV from different groundnut growing areas were reported (Paguio and Kuhn, 1973). The relatedness among these strains was determined by similarities in particle morphology, serological reactions, host range and cross-protection. They also differ in frequency of transmission through groundnut seed.

8.1 Symptoms

PeMoV produces a range of symptoms in groundnut. The widely

occurring mild mottle isolate produces mild mottle or mosaic of irregular dark green islands on young leaflets. In older leaflets the above symptoms are not obvious but can be seen in transmitted light. In some genotypes, interveinal depressions and inward rolling of margins of leaflets can occur. Infected plants are not usually stunted. The number of root nodules in diseased plants is found to be less, and the number and size of pods are reduced. Different strains of the virus cause varied symptoms (Paguio and Kuhn, 1973; Bijaisoradat *et al.,* 1988). Symptoms in groundnut caused by chlorosis and necrosis strains of PeMoV mimic the symptoms caused by TSWV (Sreenivasulu *et al.,* 1988).

8.2 Molecular diagnosis

The most common mild mottle disease caused by this virus in groundnut fields can be initially diagnosed based on interveinal depressions and inward rolling of leaflet margins. But diseases caused by strains of this virus may be confused with either PStV or TSWV infections in groundnut as they produce similar symptoms during disease development. Bioassay on French bean cv. Topcrop is useful to identify the virus. Polyclonal antiserum to this virus is produced in different laboratories and is also commercially available. PeMoV is found to be serologically distinct from other groundnut infecting potyviruses (PStV, groundnut eyespot, peanut green mosaic). ELISA is commonly used to detect the virus in both leaves and seed of groundnut (Bharathan *et al.,* 1984; Hobbs *et al.,* 1987). Dot-blot hybridization test using ^{32}P-labelled cDNA probe was developed for its detection in groundnut seed (Bijaisoradat and Kuhn, 1988). The virus was readily detected in 1 mg of infected groundnut seed tissue by this test. They claimed that this test is 8-10 times more sensitive compared to ELISA. Gillaspie *et al.* (2000) have developed IC-RT-PCR that can detect the virus in seed lots and can distinguish it from PStV.

8.3 Phylogenetic relationships

The mild mottle isolate is the most widely distributed one. Whereas the remaining strains (N, S, CLP) are rare and reported from USA only. The strains of PeMoV were initially distinguished mainly based on symptoms in groundnut and other experimental hosts and serological relationships (Bijaisoradat *et al.,* 1988). Radioactive nucleic acid probes were first used to determine the nucleotide sequence homologies of PeMoV and PStV (Bijaisoradat and Kuhn, 1988). Eight strains (AR, CS, DE, IN, LB, M, N, NC; Bijaisoradat *et al.,* 1988) of PeMoV have similar nucleotide sequence homology. On the other hand, PStV showed an average of 50% homology with several strains of PeMoV. The 3’ terminal of 1247 nucleotides of Australian isolate of PeMoV has been sequenced. PeMoV and PStV

shared a 64.4% sequence similarity in the coat protein gene and 34.6% similarity in the 3′ UTR (Teycheney and Dietzgen, 1994). Its sequence similarity with remaining groundnut infecting potyviruses was not determined. Recently Adams *et al.* (2004) have analysed the sequences of several potyviruses and suggested the criteria to discriminate between the genera and species in the family *Potyviridae.*

8.4 Management approaches

The low level transmission of the virus through the seed of not only peanut (0-8.5 %) but also a few grain legumes (cowpea, mung bean, common bean) is contributing for the primary spread of the virus. Alternate crops (cowpea, soybean, clover, peas, navy bean, French bean, white lupine) and weeds (*Desmodium, Cassia* sps.), and aphids are facilitating the survival and spread of the virus in nature (Demski, 1975). The incidence of the virus in young groundnut fields appears to be very low (<1 %). As the crop reaches maturity, the disease progresses to nearly 80% under congenial conditions that favor vector activity in the fields.

As the seed is identified as a primary source of virus inoculum, genotypes that do not transmit the virus through the seed, such as ICG 2716 (EC 76446-292), ICG 7013 (NCAC 17133) and ICG 1697 (NCAC 17090), are useful in containing the spread of the virus. These lines were used in conventional breeding programmes to transmit the non-seed transmissible trait to high yielding groundnut cultivars. The seed of advanced breeding lines from these crosses has been tested for frequency of the virus transmission. Two non-seed transmitting high yielding groundnut genotypes (ICGS 65 and ICGS 76) were identified. Usually cultivated groundnut is susceptible to PeMoV. Kuhn *et al.* (1978) have identified two groundnut genotypes (PI 261945 and PI 261946) that are tolerant to PeMoV infection in USA. Later in India also, the groundnut genotypes, ICG 5043 and NCAC 2240 were found to be tolerant with no significant effect on yield after virus infection. High yielding groundnut genotype (ICG 89336) with tolerance to PeMoV is available. *Arachis chacoense* and *A. pusilla* and wild *Arachis* sps., have been reported to be resistant to PeMoV (Demski and Sowell, 1981). However, this resistance is yet to be transferred to cultivated high yielding groundnut cultivars.

9. *CUCUMBER MOSAIC VIRUS* (PEANUT YELLOW MOSAIC)

Cucumber mosaic virus (CMV) naturally infects groundnut in northern parts of China (Xu and Barnett, 1984). The disease caused by this virus is referred to peanut yellow mosaic and it causes crop losses of upto 40%. CMV is the type species of the genus *Cucumovirus,* family *Bromoviridae.* It

is one of the most widespread plant viruses in the world that has very extensive natural and experimental host range (Palukaitis *et al.,* 1992). This virus has been successfully adapting to new hosts and environments (Roossinck, 2002). It is readily sap transmitted and by more than 75 aphid species in non-persistant manner. Further, it is reported to be transmitted through the seed of crop and weed plants and through vegetative planting materials (Hsu *et al.,* 2000). The virions of the virus are non-enveloped isometric particles measuring 26-30 nm in diameter. The coat protein of virions consists of single polypeptide of 25-26 kDa. The virus genome is positive sense, linear, tripartite ssRNA (RNA 1: 3.389 kb; RNA 2: 3.035 kb; RNA 3: 2.197 kb). In addition to 3 genomic RNAs the virions also contain sub-genomic RNA (RNA 4: 1.027 kb) representing RNA 3 and a few isolates contain satellite RNA (CMV Associated RNA or CARNA 5). The satellite RNA is known to modulate the symptom expression in certain hosts. Several strains of CMV that naturally infect different crops have been classified into two major subgroups (subgroup I and II) on the basis of serological properties and nucleotide sequence homology (Palukaitis *et al.,* 1992; Hsu *et al*., 2000). Roossinck *et al.* (1999) have further divided subgroup I into two groups (I A and I B) by phylogenetic analysis.

The CMV isolate (CMV-CA) naturally infecting groundnut in China is easily sap transmissible and transmitted by several aphid species (*Macrosiphum euphorbiae*) in a non-persistant manner. It is also transmitted through the groundnut seed up to 2-4 % (Xu and Barnett, 1984). This isolate can infect 31 plant species in 6 families by sap inoculation. The purified CMV-CA preparation contained non-enveloped, isometric particles measuring 29 nm in diameter. The virus particles contain tripartite ssRNA (1.16×10^6, 1.05×10^6 and 0.81×10^6) and a sub-genomic RNA (0.39×10^6 daltons).

9.1 Symptoms

The initial symptoms on peanut induced by CMV are chlorotic spots and upward rolling of young leaflets. Adjacent spots may coalesce to form large yellow blotches. Subsequently developed leaflets exhibit yellowing of the lamina with green stripes along the lateral veins. Occasionally, leaflets are deformed and plants are moderately stunted. The severe yellowing and mottling symptoms initially observed in young plants are not apparent on older plants.

9.2 Molecular diagnosis

Bioassays on diagnostic hosts (cowpea, cucurbits, tobacco, *Datura stramonium* and *Chenopodium* species) have been used for the initial tentative diagnosis of CMV isolates (Palukaitis *et al.,* 1992). Polyclonal and monoclonal antibodies have been produced to several CMV isolates and

they are available commercially also. ELISA based tests are optimized for routine detection of the virus on a large scale (Porta *et al.,* 1989). Radioactive and non-radioactive nucleic acid probes are used for its detection in varied plant samples by dot-blot hybridization (Palukaitis *et al.,* 1992). Either partial or complete genome sequences of CMV isolates are determined and primers have been designed for amplification of RNA 3 by RT-PCR (Rizos *et al.,* 1992; Choi *et al.,* 1999; Dietzgen *et al.,* 2001).

9.3 Phylogenetic relationships

CMV isolates vary in their host range and the type of symptoms induced by them in specific hosts. This approach is used to differentiate the CMV isolates into pathotypes (Palukaitis *et al.,* 1992). Agar gel double diffusion, ELISA and western blot analyses have been used to determine the antigenic relationships not only among the isolates of CMV but also with other members of the genus *Cucumovirus*. Numerous strains of CMV have been classified into two major sub-groups (sub-group I or DTL, WT and II or ToRS, S) on the basis of serological properties (Palukaitis *et al.,* 1992; Hsu *et al.,* 2000). Sub-group I isolates predominate in the tropics. Coat protein peptide profiling either by SDS-PAGE or HPLC has been used to differentiate the isolates of CMV (Edwards and Gonsalves, 1983). Nucleic acid hybridization-based approaches were also used for differentiating CMV strains (Piazolla *et al.,* 1979). In recent times genome (especially RNA 3) sequence data of CMV isolates is used to determine phylogenetic relationships among them (Owen *et al.,* 1990; Roossinck *et al.,* 1999). The sub-group I and II isolates in phylogenetic analysis cluster as two distinct groups. Two strains of CMV are reported to naturally infect peanut in China. Of these, CMV-CA is a predominant strain and CMV-CS is of minor importance. The latter is serologically related to *Peanut stunt virus.* Whereas CMV-CA is distinct from CMV-CS (Xu and Barnett, 1984). The predominant CMV strain from China reacted with antisera to CMV-D and CMV-CI but not with CMV-S. The nucleotide sequence analysis of these two isolates may reveal their exact identity and relationships.

9.4 Management approaches

As CMV has wide host range, more than 75 aphid species as vectors and can transmit through seed and vegetative propagules of several plants, it perpetuates well in different agro-ecosystems (Palukaitis *et al.,* 1992). The CMV-CA isolate is groundnut seed transmissible (2-4%) and thus the primary spread is probably initiated through the seed-borne virus. Aphids may contribute for its secondary spread in peanut fields. Planting with CMV-free groundnut seed reduced disease incidence. Cultural practices like mulching with transparent plastic sheets and rogueing of diseased seedlings at early stages of crop growth reduced disease incidence in China.

There was no resistance to CMV in the cultivated groundnut germplasm.

10. CONCLUSIONS

A large number of viral diseases have been reported to constrain groundnut yield, most of them are widespread, but not all are economically important. In this chapter eight important diseases caused by TSWV, GBNV, TSV, IPCV and PCV, PStV, PeMoV, CMV, and rosette complex are detailed. These viruses were characterized, various serological- and nucleic acid-based diagnostic tools developed and diverse management options have been established. At present major research emphasis in various laboratories is being continued on TSWV, GBNV, TSV, rosette complex and peanut clump. Although substantial progress has been achieved on these viruses, one major constrain is the lack of improved varieties adapted to the various agroecologies found in developing countries. Considering the fact that nearly 90% of the groundnut area is in developing countries of Asia and Africa, simple and affordable practical options, such as durable resistant varieties, are needed to farmers for sustainable management of these virus diseases. Biotechnological approaches involving marker-assisted breeding and/or genetic engineering are being pursued to hasten the development of elite disease resistant varieties to combat these diseases in various laboratories (Sharma and Ortiz, 2000; Sharma *et al*., 2002; Herselman *et al*., 2004; Popelka *et al*., 2004). At ICRISAT, groundnut cultivar JL24 was transformed with nucleoprotein gene of GBNV, TSV, GRAV and IPCV, and these events are being evaluated for resistance (KK Sharma, Personal Communication). If successful this will offer reliable resistance to these viruses that can also be bread into other groundnut cultivars through back cross breeding programs.

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