Indian Peanut Clump Virus, a Fungal Transmitted *Pecluvirus* Infecting Both Monocotyledonous and Dicotyledonous Plants in India

16

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Abstract

Indian peanut clump virus (IPCV), a member of the genus *Pecluvirus* under the family *Virgaviridae* has a wide host range, which include monocotyledonous and dicotyledonous plants. Among the diseases caused by IPCV in different crops, clump disease of peanut or groundnut is most important and one of the major limiting factors of groundnut production in sandy and loamy-sand soils in Andhra Pradesh, Tamil Nadu, Gujrat, Rajasthan and Punjab. A comprehensive research on transmission, host-range, serology and molecular characterization of this virus was carried out in India from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad. The virus is transmitted by sap, seed, and fungus (*Polymyxa graminis*). Besides groundnut, IPCV also infects pigeonpea, cowpea, chili, wheat, barley, sorghum, sugarcane and maize. Virion of IPCV has two rod-shaped particles measuring 249 and 184 nm in length and 24 nm in diameter. ELISA and immunosorbent electron microscopy revealed three serotypes of IPCV, *viz.* IPCV-Hyderabad, IPCV-Durgapura and IPCV-Ludhiana. The genome of IPCV composed of two positive sense ssRNA components, which are encapsidated by a single coat protein. Till now the complete sequence of one RNA-1 and three RNA-2 components of IPCV are available in the sequence database. Sensitive broad spectrum detection of IPCV has been demonstrated using non-radioactive nucleic acid probes. Cultural practices like soil solarization, early planting, and trap cropping with pearl millet are some measures for management of the disease.

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16.1 Introduction

A groundnut disease characterized by severe stunting and clumping of plants was first time described in India by Sundararaman in 1927. However, scientific investigation on the disease and its etiology was initiated during 1977–1978 when Dr. D.V.R. Reddy and his team from ICRISAT, Hyderabad, India noticed severe appearance of the disease in Punjab (Reddy et al. 1979). The disease appeared in patches in the field where crops were mainly grown in sandy and sandy-loam soils. The disease was also observed in groundnut plants where crops were raised in sandy soils in Andhra Pradesh, Gujarat, Tamil Nadu and Rajasthan. A similar disease was reported from Africa (Trochain 1931), where a bipartite, rigid rod-shaped soil borne virus, peanut clump virus (PCV), was reported to be responsible for causing the disease (Thouvenel et al. 1976). However, the serological study of the virus causing similar clump disease in groundnut in India revealed that it was distinct from that known in Africa and named as Indian peanut clump virus (IPCV) (Reddy et al. 1983). Later, based on the similarities on symptoms, particle morphology, mode of transmission and hybridization study indicated IPCV serotypes could be strains of PCV (Reddy et al. 1985). However, when sequence information was available for both PCV and IPCV, it was realized that there were some distinct genomic differences of these viruses from the other fungal transmitted furoviruses. Hence, a separate genus, *Pecluvirus,* was created in the family *Virgaviridae* with type species *Peanut clump virus* (https://talk.ictvonline.org/ictv-reports/ictv_9th_report/). PCV and IPCV are two distinct species under the genus *Pecluvirus* and they differ among themselves in spatial distribution, host-range, symptomatology, serology and nucleotide homology. Information on IPCV generated through research in India has been reviewed and summarized in following sections.

16.2 Virus Morphology

Nolt et al. (1988) observed virus particle in electron microscope as typically bipartite non-enveloped rigid rod with two predominant sizes of 190 × 24 nm and 245 × 24 nm. Both the genomic components are encapsidated by a single type of coat protein. Rao et al (2002) observed presence of ridgid rod shaped paticles measuring 250 × 20 nm from *Chenopodium amaranticocor* showing chlorotic lession after inoculation of virus from sugarcane leaves showing red leaf mottle symptom.

16.3 Host Range

IPCV is known to cause disease in several commercial crops like groundnut, pigeonpea, cowpea, chili, wheat, barley, sorghum, sugarcane and maize. Besides, IPCV has experimental host plants like *Nicotina benthamina*, *N. glutinosa, Phaseolus vulgaris* cv. Topcrop, *Vigna unguiculata* cv. Pusa Komal and *Chenopodium amaranticolor*. Monocotyledonous weeds such as *Cynodon dactylon* and *Cyperus rotundus* were reported as source of primary inoculums when main crops are not present in the field (Nolt et al. 1988).

16.4 Symptom Variation in Different Hosts

In case of groundnut, major symptom includes stunting, clumping and smalling of leaves, dark green leaf colour and small size of infected pods (Fig. 16.1). In case of wheat, plants became rossatte like with malformed spikes, which often did not emerge from the flag leaf and they contained few shrivelled seeds. In barley, severe stunting and leaf chlorosis was caused by IPCV. With the aging of the plants, the leaves became necrotic, only few infected plants reached the maturity and produced small spikes. Stunting was the prominent symptom in pearlmillet. In sugarcane, Rao et al. (2002) reported that a PCV isolate was associated with the red leaf mottle disease based on serology and electron microscopy. However, as PCV was reported to be present only in Africa, it is assumed that in India, the red leaf mottle of sugarcane may be associated with IPCV. In sugarcane, along with slight stunting, various degrees of mottling, which later turned into red streaks of varying intensities was observed upon infection of the virus (Rao et al. 2002). Necrotic local lesions were produced in *Chenopodium amranticolor* while chlorotic local lesions were produced in cowpea cv. Pusa Komal. Systemic chlorotic ring spot and leaf

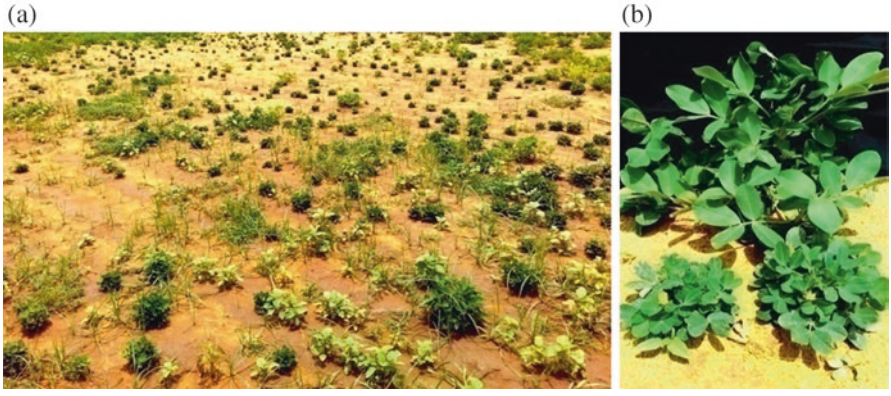

Fig. 16.1 Groundnut plants showing clump disease in field at Bikaner District, Rajasthan during 2014. (**a**) High incidence of the disease in field, (**b**) comparison of symptomatic (small and bushy) and asymptomatic plants

deformation developed in *Nicotiana benthamiana* infected by IPCV. Systemic mosaic was the main symptom in *N. glutinosa.* Veinal chlorosis and veinal necrosis developed in French bean.

16.5 Impact of the Disease

Peanut clump disease causes significant losses in groundnut crops in West Africa and in the Indian subcontinent (Reddy et al. 1999). Globally, annual losses in peanut due to the disease have been estimated to exceed US$ 38 million (Reddy et al. 1999). Total yield loss was reported when IPCV infection in groundnut occur in an early crop growth stage. Reduction of grain yield occur upto 60% in case of late infection. Besides groundnut, wheat and barley crops were also shown to be naturally infected by IPCV under field conditions. It has been shown that in case of wheat infected with Hyderabad isolate of IPCV, vegetative biomass was reduced up to 33%, which resulted into 36% grain loss (Delfosse et al. 1999). Early infection of Hyderabad isolate of IPCV (IPCV-H) in wheat resulted in severe stunting with dark green appearance of plants and chlorotic streaks on the youngest leaves, which turned necrotic as the plants aged. If wheat plants were infected in later stage of crop growth, their maturity is delayed and such infected plants can readily be identified in the fields because of their dark green appearance. The yield reduction in IPCV-H infected wheat was as high as 58% (equivalent to a yield loss of 1800 kg/ha) (Delfosse et al. 1999).

16.6 Transmission and Perpetuation of the Virus

IPCV was shown to be transmitted by sap, seed, and fungus. Soil-borne fungal pathogen, *Polymyxa graminis*, transmits the virus to dicotyledonous crops, however, in the roots of dicotyledonous plants it does not extensively colonize, which is evident from either absence or presence of less number of sporosori (resting spore clusters or cystosori) in their roots (Ratna et al. 1991; Delfosse et al. 1996; Legrève et al. 1999; Legrève et al. 2000). It has been demonstrated that roots of naturally virus-infected groundnut plants did not contribute to spread of the disease when inoculated into sterile sand, where as roots of sorghum and pearl millet plants infected with IPCV could induce the disease and serve as source of inoculum (Thouvenel et al. 1988; Ratna et al. 1991; Delfosse et al. 1996). This indicates that IPCV can be transmitted to dicotyledonous plants through fungal vector by a matter of chance but roots of such infected plants can not contribute as a source of inoculum for further fungal transmission. Hence, dicotyledonous plants served as 'fortuitous' hosts for IPCV and they do not contribute to building up of inoculum in soil. In contrast, maize, pearl millet, and sorghum are the monocotyledonous hosts which are 'preferred' hosts for *P. graminis* as the fungus can multiply in them and produce more number of sporosori in their roots (Ratna et al. 1991; Delfosse et al. 1996; Legrève 1999). As IPCV also able to multiply in these monocotyledonous hosts, they are supposed to play an important role in the perpetuation and spread of virus inoculum under field conditions (Reddy et al. 1999). However, *P. graminis* was

rarely observed in roots of wheat plant and was not detected in barley, thus their role in virus perpetuation has not yet been established.

Besides fungal transmission, IPCV could also be transmitted through seeds from the infected plants. In case of groundnut, under natural field condition, seed transmission varied from 3.5 to 17%, depending on the genotype. However, when seeds from infected groundnut plants were used, the transmission efficiency was increased to 48–55%. In case of monocotyledonous plants, seed transmission was demonstrated with IPCV-H isolate, which was shown to be transmitted by seed in finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), and pearl millet (*Pennisetum glaucum*) with 5.2%, 9.7%, and 0.9% efficiency, respectively. In case of wheat, seed transmission efficiency was 0·5–1·3% (Delfosse et al. 1999). No seed transmission was observed in sorghum (*Sorghum bicolor*) (Reddy et al. 1998).

16.7 Epidemiology

High rainfall, allows the free movement of motile zoospore of *P. graminis* and thus increases the transmission of the virus, which results in higher incidences of the disease. A weekly rainfall of 14 mm is sufficient for *P. graminis* to initiate infection. It has been shown that groundnut crops either grown during the post-rainy season or crops grown in the rainy season but sown well beyond the onset of monsoon rains mostly escaped the disease (Reddy et al. 1988). Temperature is another important factor for IPCV infection. Temperature ranged from 23 to 30 °C, which is prevailing during the rainy season were found to be conducive to natural virus transmission (Delfosse et al. 2002). However, under mechanical inoculation condition, IPCV causes infection on wheat at a wider temperatures between 15 and 30 °C (Reddy et al. 1988), indicating the wider temperature adaptation of this virus.

16.8 Serological Properties

Different IPCV isolates were collected and grouped according to the serological properties. The name of IPCV isolates given based on the places from where they were first reported in India. Antiserum was prepared and reactivity was tested through ELISA and ISEM. Based on the serological properties, three distinct serotypes of IPCV were identified, IPCV-D (Durgapura isolate, Rajasthan), IPCV-H (Hyderabad isolate, Andhra Pradesh) and IPCV-L (Ludhiana isolate, Punjab) (Reddy et al. 1983; Nolt et al. 1988).

16.9 Molecular Properties

16.9.1 Genome Size and Sequence

IPCV genome is bipartite positive sense single stranded RNA, which constitutes 4 % of virion mass by weight. Only one RNA-1 genome sequence of Hyderabad isolate of IPCV is available (Miller et al. 1996, accession no. X99149), while three

sequences of RNA-2 of IPCV are available in sequence database (Naidu et al. 2000; accession nos. AF239729, AF447396, AF447397). The RNA-1 is 5841 nucleotides and RNA-2 is 4290–4507 nucleotides long.

16.9.2 Genome Organization

RNA-1 sequence of Hyderabad isolate of IPCV have M_7G capping at 5′ end followed by untranslated region (UTR) of 132 nucleotides and code for three proteins (P131, P191, and P15). The protein products of P131 and P191 are involved in replication of the viral genome. P131 is coded by nucleotide sequence from 133 to 3525 and acts as methyl transferase, whereas P191 encoded by translational read-through of UGA stop codon of P131 protein from the nucleotides 133 to 5103, functions as helicase as well as RNA-dependent RNA polymerase. Protein P15 is encoded by a sub-genomic RNA representing nucleotide coordinate 5168 to 5542 of RNA-1 and it appears to be a suppressor of post-transcriptional gene silencing. After P15 ORF there is an untranslated region of 299 nucleotides, which ends with 3′ –OH group.

RNA-2 of IPCV starts with M_7G capping at 5′ end and ends with 3′ –OH group. RNA-2 of IPCV codes for five proteins related with capsid formation, vector transmission and movement of the virus within the host plant. RNA-2 have UTR of variable lengths; 388 nucleotides in Ludhiana isolate (L), 394 nucleotides in Durgapura isolate (D) and 502 nucleotides in Hyderabad isolate (H). The first ORF after the 5′ UTR codes for approximately 24 K Dalton coat protein. The ORF of coat protein is 600 nucleotides in L isolate, 663 nucleotides in D isolate and 627 nucleotides in case of H isolate. The next ORF codes for a vector transmission factor (P40/P39/P38) and translated through the sub-genomic RNA of size 1065 nucleotides (991-1065) in L isolate, 1020 nucleotides (1129-2148) in H isolate and 909 nucleotides (1058-1966) nucleotides in Durgapura isolate. Next three ORFs constitute the triple gene block, which involved in movement of IPCV. After triple gene block, there is a 3′ UTR of 280 nucleotides in L isolate 290 nucleotides in D isolate and 275 nucleotides in H isolate.

16.9.3 Sequence Comparison and Phylogeny

(a) Untranslated Region

5′ and 3′ UTR of RNA-1 are 132 and 299 nucleotides in length, respectively, whereas the RNA-2 has more variable UTR of 388 to 502 nucleotides at 5′ end and approximately 300 nucleotide at 3′ end. Within the 3′ UTR of RNA-2, approximately 100 terminal nucleotides are conserved in all the three isolate, which was used for the development of probe for detection of IPCV (Wesley et al. 1996).

(b) Coat Protein

Coat protein is coded by RNA-2. Nucleotide identity matrix of coat protein sequence retrieved from NCBI of all the isolates of IPCV, PCV and one species of tobacco rattle virus revealed that IPCV isolates are 52–59% similar among themselves, whereas they show 40–61% similarity with the PCV. Whereas, with TRV they show similarity up to 34–35% (Wesley et al. 1994).

(c) Complete RNA-2 Based Phylogeny

In most of the members of the family *Virgaviridae*, coat protein is used as conserved protein for phylogenetic analysis. But in case of IPCV, coat protein sequence is highly diverse and hence complete sequence of RNA-2 is used for the phylogenetic analysis. The Hyderabad and Durgapura isolates of IPCV are more similar than the Ludhiana isolate.

16.10 Management of the Virus

The efforts to develop management practices against IPCV were focused on identifying the resistant sources, and manupulating the cultural practices. Nine thousand *Arachis* germplasm lines were tested to find out resistance against IPCV, but none found resistant (Delfosse 2000). Application of soil biocides and soil solarization to control the disease although found effective, but they were either hazardous or uneconomical (Dhery et al. 1975; Reddy et al. 1999). It has been shown that if sowing of the groundnut crop was done before the onset of monsoon rains and the crop is raised with judicious irrigation, then it can effectively reduce the disease incidence (Delfosse 2000). Use of pearl millet as a trap crop was found successful at different sites in India (Delfosse et al. 1997). As *P. graminis* multiplies intensively in monocots, crop rotation with monocotyledonous plants should be avoided to reduce population of the fungal vector in the soil.

16.11 Concluding Remarks

Pecluviruses were discovered in peanut and so far the genus Pecluvirus contains only two virus species, IPCV and PCV. The geographical distribution of IPCV and PCV is restricted only in India and West Africa, respectively. IPCV is a soil borne and seed-transmitted virus with a broad host range, however, its spread and survival depend on critical interactions of the fungus vector, *P. graminis* with the type of host plant species. It is hypothesized that IPCV possibly coevolved with wild grasses and cereal crops such as millets and sorghum in the tropical and subtropical areas. In monocotyledonous plants, IPCV causes little damage or do not show any symptom but these crops support multiplication of *Polymyxa graminis* and thus may promote virus accumulation. When IPCV is transmitted to the susceptible dicotyledonous

crop such as peanut, it causes economically important disease. However, as *P. graminis* does not reproduce in peanut, the IPCV inoculum in the soil is expected to reduce in the peanut field. IPCV and PCV are quarantine viruses to many countries. Rate of seed transmission of IPCV is higher than any of the seed transmitted viruses infecting peanut. IPCV is also transmitted through seeds of millets, wheat, and maize. Therefore, the seeds of these crops specially from the infected regions pose a risk of introduction of IPCV in the new geographical regions. IPCV has unique genome organization that the CP gene is located at the 5' end of RNA2 genome, whereas, in the other members of the family *Virgaviridae*, CP is located at the 3′ end of the genome. A comparison of CP gene among the three RNA2 sequence of IPCV indicated high degree of variability in this ORF, which is contrasting with other members of the family *Virgaviridae*, as CP gene sequence is highly stable within a species. Due to the availability of limited sequence information on IPCV isolates, it is difficult to comprehend the diversity within IPCV, however, it is possible that there could be the existence of new species within the genus *Pecluvirus* in India. The infectivity of the cloned DNAs of IPCV has not yet demonstrated. IPCV having hosts in both mono- and dicotyledonous plants and bipartite genome organization, is attractive for developing virus based vector for inducing gene silencing in plants.

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