Rate of Transmission of Indian Peanut Clump Virus to Groundnut by Mechanical Inoculation

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'Clump' is one of the major viral diseases of groundnut (*Arachis hypogaea*) caused by the Indian peanut clump virus (IPCV) in the Indian subcontinent (Nolt et al. 1988). A similar disease in Africa is caused by peanut clump virus (PCV) (Thouvenel and Fauquet 1981). Both PCV and IPCV belong to the genus *Pecluvirus*, and they have similar physical, biological and transmission properties, but their coat proteins are highly variable. Various isolates of IPCV and PCV occur in endemic regions (Nolt et al. 1988). IPCV and PCV are transmitted through seed and by a root endoparasite, *Polymyxa graminis*. Several serologically distinct isolates of PCV and IPCV were identified in Asia and Africa.

Clump disease occurs in patches in fields. The disease recurs when groundnut and certain IPCV-susceptible cereal hosts like pearl millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) are grown regularly (Delfosse et al. 1999). Durable resistance to clump in groundnut germplasm is lacking. Although several thousand groundnut genotypes were screened for clump resistance in experimental sick plots, none of these were consistently resistant or tolerant to IPCV (Reddy et al. 1988). Genotypes that showed resistance (no infection) or low disease incidence in one trial showed severe infection in subsequent trials at the same location. The variation in resistance/tolerance reaction in genotypes in the sick plots was due to uneven distribution of virus inoculum in the fields, which depends on the germination of resting spores of *P. graminis* and environmental conditions (Reddy et al. 1988). A reliable virus inoculation procedure is essential to accurately evaluate groundnut genotypes for IPCV resistance. Although IPCV can be transmitted by mechanical sap inoculation, it seldom was used for resistance screening probably due to low infection rate achieved by this method. In this study, rate of IPCV [Hyderabad isolate (H)] transmission to groundnut by mechanical inoculation was assessed using the virus infected leaf sap and purified virus preparations as inoculum.

Table 1. Transmission of IPCV-H after mechanical inoculation with sap extracts prepared from virus-infected French bean.

	Groundnut cv JL 24					French bean cv Topcrop				
Date of inoculation	Incubation period[1]	Infected/ Tested[2]	Infection[3] (%)	Temperature[4] Max	Temperature[4] Min	Days to infection[1]	Infected/ Tested[2]	Infection[3] (%)	Temperature[4] Max	Temperature[4] Min
03/09/04	18	6/7	85	30.0	21.0	6	6/6	100	29.4	21.4
21/09/04	19	13/20	65	31.0	21.0	5	9/10	90	30.8	21.7
23/09/04	21	9/14	64	31.0	21.0	6	5/5	100	30.4	21.9
01/10/04	20	16/17	94	30.5	20.0	5	10/10	100	30.4	21.1
12/10/04	18	12/20	60	30.0	18.0	5	14/15	93	30.9	19.7
15/10/04	18	14/20	70	29.5	18.4	6	9/10	90	29.5	15.7
26/10/04	21	6/11	55	29.3	18.0	5	10/10	100	29.0	18.7
29/10/04	20	14/17	82	29.6	17.9	6	15/15	100	28.9	19.2
03/11/04	21	14/25	56	30.0	16.1	6	10/10	100	28.6	15.9
06/12/04	23	0/25	0	29.6	11.3	5	3/5	60	29.2	10.1
13/12/04	21	9/24	37	29.6	12.4	5	9/10	90	29.2	11.0
29/12/04	23	2/6	33	29.7	13.6	6	14/15	93	28.9	15.2
31/12/04	23	14/24	58	29.8	14.0	6	10/10	100	29.3	15.4
10/01/05	21	13/26	50	30.0	15.5	6	4/5	80	30.0	11.6
17/01/05	20	14/26	54	29.3	16.8	6	9/10	100	29.7	14.8
24/01/05	22	21/28	75	30.3	16.5	7	9/10	90	30.5	19.1
Mean	20.5	177/286	62	29.9	16.9	5.5	144/156	92	29.6	17.0

1. Maximum number of days at which all the virus-infected plants showed symptoms.
2. Number of plants.
3. Infection confirmed by DAS-ELISA.
4. Mean temperature (°C) recorded during days to infection.

For the inoculum preparation, 0.05 M potassium phosphate buffer, pH 7 containing 0.1% (v/v) β-mercaptoethanol was used (referred as inoculation buffer). French bean (*Phaseolus vulgaris*) cultivar Topcrop at cotyledonous leaf stage and groundnut cultivar JL 24 at three-leaf stage were used for inoculation. Both these cultivars were highly susceptible to IPCV infection. Prior to inoculation test plants were kept in dark for 12–16 h. Test plants were dusted with carborandum (600 mesh) and freshly extracted inoculum was immediately applied onto the leaves with a double layer muslin-cloth pad. Inoculated leaves were washed with distilled water and covered with sheets of paper and kept in dark overnight. They were maintained in greenhouse chambers fitted with air-coolers to lower the day temperature (27–35°C, depending on the external temperature), which were operated during daytime only. Plants were regularly monitored for symptoms and tested for the virus in leaf samples (1:20 w/v) by DAS-ELISA (double antibody sandwich - enzyme-linked immunosorbent assay) using IPCV-H immunoglobulins as described by Nolt et al. (1988).

The IPCV-H infected groundnut seed stored at –70°C was used as initial virus inoculum source. In a pre-chilled mortar and pestle, seed material (1:10 w/v) was macerated in chilled inoculum buffer and immediately inoculated to French bean. Veinal necrosis symptoms, typical of IPCV infection, developed 4–7 days after infection. This was used as the virus source for subsequent experiments. Leaf sap extract (1:10 w/v) prepared from IPCV-H infected French bean was inoculated to 16 batches of French bean and groundnut plants raised in growth chambers on different dates from September 2004 to January 2005 (Table 1). Plants were monitored for symptoms up to 30 days after infection, and they were assayed for IPCV-H by DAS-ELISA. French bean plants were readily infected with the virus in all these experiments. Infected plants showed typical symptoms within 5–7 days after infection. Except on one occasion, 80–100% of the inoculated plants were infected with the virus, with a

Table 2. Transmission of IPCV-H to groundnut cultivar JL 24 using inoculum from virus-infected groundnut leaves.

Date of inoculation	Days to infection[1]	Infected/Tested[2]	Infection[3] (%)	Temperature[4]	
				Max	Min
18/11/04	21	5/20	25	29.2	11.5
19/12/04	23	9/26	35	29.7	13.5
04/02/05	19	19/20	95	33.2	15.4
11/02/05	18	17/24	71	34.4	16.7
Mean	21	50/90	55	31.6	14.2

1. Maximum number of days at which all the virus-infected plants showed symptoms.
2. Number of plants.
3. Infection confirmed by DAS-ELISA.
4. Mean temperature (°C) recorded during days to infection.

Table 3. Infection in groundnut cultivar JL 24 after inoculation with partially purified IPCV-H preparations.

Date of inoculation	Incubation period[1]	Dilution	Infected/Tested[2]	Infection[3] (%)	Temperature[4]	
					Max	Min
20/01/05	28	1:100	4/5	80	31.7	16.3
		1:1000	2/5	40		
		1:5000	1/5	20		
03/02/05	26	1:100	4/4`	100	33.3	16.3
		1:1000	2/5	40		
		1:5000	1/5	20		
15/02/05	25	1:100	8/9	89	34.7	17.4
Mean	26		22/38	57	33.2	16.6

1. Maximum number of days at which all the virus-infected plants showed symptoms.
2. Number of plants.
2. Infection confirmed by DAS-ELISA.
3. Mean temperature (°C) recorded during days to infection.

mean infection of 92% for the entire experiment (Table 1). The sap inoculated groundnut plants took 18–23 days to develop symptoms; infection in most experiments was 50–75%, with a mean infection rate of 62% for the entire experiment (Table 1). When the groundnut plants were inoculated with sap extract prepared from the virus-infected groundnut leaves, 23–90% of the test plants were infected, and it took 18–23 days to develop symptoms (Table 2). Groundnut plants were also inoculated with partially purified IPCV-H preparations made from 100 g virus-infected French bean leaf tissue using the procedure described by Nolt et al. (1988). The partially purified virus pellets were dissolved in 5 ml of 0.02 M sodium borate, 0.03 M potassium phosphate buffer, pH 8.3, containing 0.3 M urea, and diluted to 1:100, 1:1000 and 1:5000 in inoculum buffer and applied onto the groundnut plants. Test plants inoculated with 1:100 dilution preparations showed 80–100% infection in three separate experiments, whereas those inoculated with 1:1000 and 1:5000 dilutions showed 20–40% infection (Table 3).

The night temperature seems to have an effect on IPCV-H infection in groundnut plants. Less than 40% of the inoculated groundnut plants showed infection when the night temperature was 12–14°C, and no infection resulted when the temperature was <12°C (Table 1). During the same period, there was no difference in percentage infection in French bean, but when the night temperature dropped below 11°C, only 60% infection resulted in the test plants (Table 1). Further studies are necessary to understand the effect of temperature on IPCV infection in groundnut.

This study showed that using French bean as inoculum source, IPCV-H could be efficiently transmitted to groundnut by mechanical sap inoculation and the virus has about three weeks incubation period in groundnut. This method is convenient and allows reliable screening of elite groundnut germplasm for resistance to various IPCV and PCV isolates in relatively short period.

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