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Indian peanut clump virus (IPCV) infection on wheat and barley: symptoms, yield loss and transmission through seed

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Wheat and barley crops were shown to be susceptible to Indian peanut clump virus (IPCV) under field conditions. In wheat, the Hyderabad isolate of IPCV (IPCV-H) induced symptoms resembling the rosette caused by soil-borne wheat mosaic virus, and these were apparent only three weeks after emergence. Early-infected plants were severely stunted and dark green, with chlorotic streaks on the youngest leaves, which turned necrotic as the plants aged; most of these plants died. Late-infected plants were also stunted and were conspicuous in the field because of their dark green appearance as a result of delayed maturity. The virus was detected by ELISA and nucleic acid hybridization in all plants with symptoms. These plants usually produced fewer tillers than healthy ones. Spikes were malformed, often did not emerge from the flag leaf, and they contained few, shrivelled seeds. Grain yield was decreased, on average, by 58%. In barley, IPCV-H caused severe stunting and general leaf chlorosis. As the plants aged, the leaves became necrotic and the few infected plants that reached maturity produced small spikes. IPCV-H antigens were detected by ELISA in every wheat seed from infected plants and the virus was transmitted through wheat seed at a frequency of 0·5–1·3%. Storage at 4°C for more than a year did not affect seed transmission frequency. The virus was detected in leaves and roots of seed-transmitted plants. Seed transmission was not detected in barley. The Durgapura isolate (IPCV-D) was detected in wheat crops (cv. RR-21) at 3 different locations in Rajasthan State, India. Infected plants showed reduced growth without any overt symptoms.

Keywords: Arachis hypogaea, barley, Indian peanut clump virus, peanut, seed transmission, wheat

Introduction

Peanut clump disease is caused by viruses of the genus *Pecluvirus* (Torrance & Mayo, 1997). The disease occurs naturally in peanut or groundnut (*Arachis hypogaea*) in West Africa (Thouvenel *et al*., 1988) and the Indian subcontinent (Reddy *et al*., 1988; Mathur & Sobti, 1993; Delfosse *et al*., 1995a). Annual losses caused by clump disease in peanut globally have been estimated to exceed US$ 38 million (Reddy *et al*., 1999). The virus isolates that cause clump disease in West Africa and the Indian subcontinent are referred to as peanut clump virus (PCV) and Indian peanut clump virus (IPCV), respectively. IPCV isolates are named after the place where they were first reported in India, and fall into three distinct serotypes, 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). All the currently known members of pecluviruses are seed- and soil-transmitted (Reddy *et al*., 1988; Konaté & Barro, 1993) and have bipartite, positive-sense RNA genomes (Reddy *et al*., in press). IPCV was shown to be transmitted by the fungus *Polymyxa* sp. (Ratna *et al*., 1991) and PCV is suspected to have the same vector. IPCV and PCV have extremely wide host ranges which include many monocotyledonous plants (Ratna *et al*., 1991; Delfosse *et al*., 1996). In most production systems, peanut is either grown in rotation or as a mixed crop with cereals such as maize, millet or sorghum. Clump disease occurs at a fairly high incidence in Rajasthan, where ≈ 250 000 ha of peanut are rotated with irrigated wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) crops, grown during the post-rainy season. However, the economic importance of IPCV to these crops has so far not been investigated.

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Recently, IPCV was shown to be seed transmitted in three millets (Reddy *et al.*, 1998). This study was therefore undertaken to investigate symptoms, crop losses and seed transmission in wheat and barley caused by IPCV infection.

Materials and methods

Cultural practices in wheat and barley cultivation

The study was conducted during three consecutive post-rainy seasons (1994–95, 1995–96 and 1996–97) on the ICRISAT-Patancheru farm (near Hyderabad) in an IPCV-H-infested field. The soil was a sandy alfisol with a pH (H_2O) close to neutral. Certified seed of wheat (cv. RR-21) and barley (cv. RD 103) was treated with thiram at 3 g kg^{-1} seed and sown (≈ 100 kg ha^{-1}) in 1 m broad beds with four rows per bed during the last week of November or first week of December. Di-ammonium phosphate (80 kg ha^{-1}) was applied at the time of sowing. Urea (70 kg ha^{-1}) was applied as a top dressing at 2 weeks and 2 months after emergence. The crop received two 30-mm irrigations each week. Barley was only grown in the 1994–95 season.

Sample collection and analysis of yield components

During the 1994–95 season, one week after emergence, leaf samples were collected from wheat plants with and without symptoms, grown in the areas of the field where clump disease had occurred in peanut crops during the rainy season. One month after sowing, and subsequently at various stages of the crop growth, plants exhibiting severe stunting and dark green leaves with chlorotic stripes, as well as healthy-looking plants, were collected from 4 IPCV-H-infested patches, and tested for the presence of the virus by enzyme-linked immunosorbent assay (ELISA) and by nucleic acid hybridization assay with a nonradioactive probe. A limited number of plants were also assayed by immunosorbent electron microscopy (ISEM) and bioassay. Roots of 35 plants that tested positive in ELISA were examined for the presence of *Polymyxa* sp. During the 1994–95 season, only a few infected plants were recorded in each

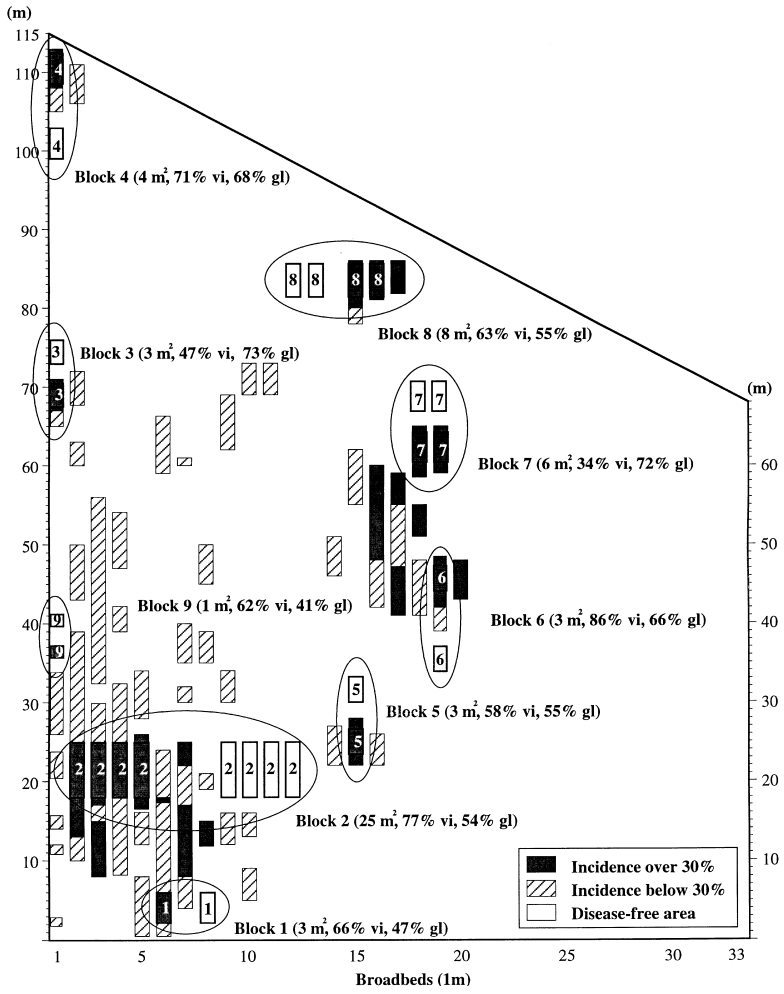

Figure 1 Clump disease distribution in wheat crop grown during the 1995–96 post-rainy season in a field naturally infested with the Hyderabad isolate of Indian peanut clump virus (IPCV-H). The entire field was under wheat cultivation and the virus incidence based on visual observation and ELISA is shown. For the assessment of yield loss caused by virus infection under field conditions, 9 replication blocks were selected, each containing an infested and a healthy plot. In each block, an equal area of wheat was harvested from both infested and healthy plots. For each block, the area that was harvested (m^2), the virus incidence (% vi) in the infested plot, and the grain yield loss (% gl) (infected vs. healthy) are indicated in brackets.

infested patch, and it was therefore difficult to study the effect of IPCV-H infection on yield on an area basis. Therefore, spikes were collected from barley and wheat plants that tested positive in ELISA on the flag leaf, and from healthy plants, to assess the number and weight of kernels per spike and the weight of 1000 kernels.

High disease incidence during the 1995–96 post-rainy season allowed the yield loss caused by IPCV-H infection to be studied on an area basis. The experiment was conducted in a randomized block design with 9 replicates for each treatment (infested and healthy) (Fig. 1). The plot size was identical within a block but varied from 1 to 25 m^2 for each block (according to the size of the infested patch), with an average size of 6·2 m^2. The infested patches chosen for the analysis showed a uniform distribution of infected plants with over 30% incidence. IPCV-H incidence was measured by visual symptoms and ELISA tests. For measurements and sample collection in infested plots, care was taken to leave a border of infected wheat plants between infested and healthy areas. The healthy plot chosen for yield comparison was selected in a disease-free area located as close as possible to the corresponding infested plot. After measuring plant height (10 plants per plot) and plant population per m^2, wheat was harvested manually from the whole plot. Various yield components were measured for each plot (Table 1). Small, shrivelled, dark-coloured seeds were considered as immature and were separated from mature seeds by a sieve with 3-mm holes to facilitate the determination of immature seed weight. During the 1996–97 post-rainy season, only the number and weight of kernels per spike and the weight of 1000 kernels were studied for spikes collected from infected and healthy plants.

Surveys of wheat crops in Rajasthan

Surveys for IPCV incidence in wheat crops were undertaken in the Boraj, Durgapura and Rampura regions in the Jaipur district of Rajasthan. During the 1994–95 and 1995–96 post-rainy seasons, the wheat cultivars in the fields surveyed were RAJ 3077 and RAJ 1482, and during the 1996–97 post-rainy season the cultivar was RR-21. Samples collected during the surveys were tested by ELISA for the presence of IPCV-D. To ascertain if IPCV-D infection occurred in plants that tested negative by ELISA, the plants were transferred to sterile soil in pots and maintained at a temperature of 25–30°C, which is known to favour IPCV multiplication.

ELISA and ISEM

The samples were assayed by the penicillinase-based (Sudarshana & Reddy, 1989) double-antibody sandwich ELISA procedure, using IPCV-H or IPCV-D antisera, similar to that described by Reddy *et al*. (1998). Results were recorded after 30 min to 1 h of substrate reaction time. Readings were considered positive if the difference in the absorbance value at 620 nm between infected and control sample exceeded 1 OD unit. For immunosorbent electron microscopy (ISEM) IPCV particles were trapped and decorated following the procedure described by Nolt *et al*. (1988).

Nucleic acid hybridization assay

Cloned cDNA of IPCV-H RNA-1, corresponding to the sequence from position 5,099–5,841, and labelled with digoxigenin, was used as a probe to detect IPCV-H in

Table 1 Effect of Indian peanut clump virus (Hyderabad isolate, IPCV-H) on yield components of the wheat cultivar RR-21 during the 1995–96 post-rainy season at ICRISAT-Patancheru

	Healthy[a]			Infected				
Parameter	Mean[b]	s.d.	Range	Mean[b]	s.d.	Range	% Loss	*F* *P*-value
Plant height[c] (cm)	99	5	91–105	36	10	27–49	64	<0·001
Population × 1000 ha^{-1}	821	335	435–1540	657	305	263–1080	20	0·021
Spikes per m^2	368	76	236–526	269	93	165–411	27	0·010
Total biomass (kg/ha)	8045	1813	4919–10610	4685	1886	2660–8506	42	<0·001
Straw yield[d] (kg/ha)	3662	924	2042–4802	2509	1111	1327–5012	31	0·014
Grain yield (kg/ha)	3121	722	2107–4067	1305	576	644–2360	58	<0·001
Harvest index	0·39	0·04	0·33–0·44	0·28	0·04	0·19–0·3	–	<0·001
Test weight (g L^{-1})	835	20	795–864	780	27	736–821	7	<0·001
1000-kernel weight (g)	33	3	28–38	28	3	23–33	14	<0·001
Immature grains (%)	7·0	4·9	2·5–18·6	21·9	7·8	12–35	–	<0·001
IPCV-H incidence				63	15	34–86		

[a]These plots showed apparently healthy plants and were located in areas known to be disease free.
[b]Means and standard deviations from 9 replicated plots varying from 1 to 25 m^2 with an average of 6·2 m^2.
[c]Mean height of 10 plants randomly measured in each plot.
[d]The straw weight did not include the weight of husks and rachis.

Figure 2 Symptoms caused by IPCV-H infection on wheat, cv. RR-21 (a, b, c, d) and barley, cv. RD-103 (e, f). (a) An early-infected wheat plant showing severe stunting compared to healthy plants. (b) An early-infected wheat plant showing rosette, chlorotic streaks and dark green leaves. (c) Chlorotic streaks on the flag leaf of a late-infected wheat plant. (d) The startling yield difference between (left) healthy and (right) infected plants. (e) Chlorotic leaves and early senescence in an early-infected barley plant. (f) Chlorotic streaks on the flag leaf of a late-infected barley plant.

leaves of wheat plants as previously described (Wesley *et al*., 1996).

Biological assays

Leaf extracts from wheat plants infected with IPCV-H were mechanically inoculated onto carborundum-dusted leaves of *Phaseolus vulgaris* (cv. Topcrop), a good diagnostic host for IPCV (Reddy *et al*., 1998). In experiments to assess whether inoculation of IPCV-H under laboratory conditions could reproduce the symptoms observed under field conditions on wheat, inoculum prepared from *P. vulgaris*, containing $1\,g\,L^{-1}$ diatomaceous earth (grade II, Sigma D-5509, Sigma Chemicals, St. Louis, MO, USA), was sprayed with an air-brush onto roots of one-week-old wheat seedlings. These were then transplanted into pots containing sterile sand and maintained in a glass-house at 25–30°C. Fifteen days after inoculation the plants were scored for symptoms and assayed by ELISA.

Determination of seed transmission frequency and viability of wheat and barley seed from infected plants

Kernels from plants that tested positive and from those that tested negative in ELISA were stored at 4°C until

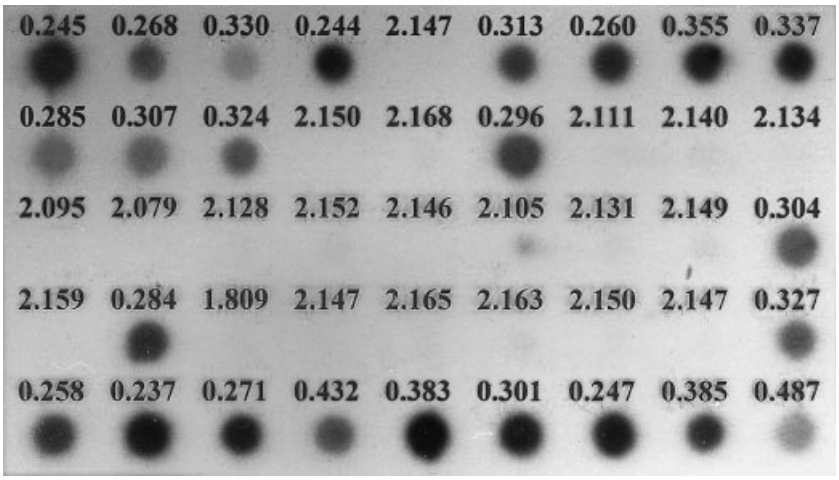

Figure 3 Detection of Indian peanut clump virus, Hyderabad isolate (IPCV-H), in extracts of wheat leaves. Samples of 50 μL of total RNA from wheat leaves spotted on the membrane and hybridized to digoxigenin-labeled probe. Samples of the same plants were tested for the presence of IPCV-H coat protein by the penicillinase-based system of enzyme-linked immunosorbent assay (ELISA), and the absorbance values (A620) are shown: high absorbance values indicate that no conjugate was bound whereas low values mean that the conjugate was bound, i.e. IPCV-H was present.

used. Kernels from each individual spike were pooled and soaked overnight in sterile distilled water to facilitate grinding. Presence of viral antigen in seeds was assessed using the extract from a single seed for each well of the ELISA plates. To eliminate any externally contaminating virus, seeds from infected plants were repeatedly soaked in $10\,g\,L^{-1}$ Na_3PO_4 solution and rinsed several times with distilled water. Kernels from a certified seed lot were also included in ELISA tests.

To determine the frequency of seed transmission of IPCV-H in wheat and barley and the effect of virus infection on germination, seeds from individual infected plants and from healthy plants were germinated on moist paper towel in Petri dishes (a separate dish for the seeds from each individual spike). Germination percentage was recorded after 10 days. Seedlings were transplanted into pots containing sterile sand and maintained in a growth chamber at 25–30°C, using modified Hoagland nutrient solution. Two-week-old seedlings were processed by ELISA, initially in groups of five for each well of the ELISA plate. Individual plants were tested from groups that gave a positive reaction. Randomly chosen ELISA-positive wheat seedlings were also tested by infectivity assays.

Detection of *Polymyxa* sp.

Polymyxa infection in wheat and barley roots was assessed under natural conditions by examining root samples for the presence of cystosori by light microscopy (Maraite *et al*., 1988).

Data analysis

The effect of IPCV-H infection on yield components was evaluated by analysis of variance for the data collected during the 1995–96 season and by the Mann–Whitney rank sum test for the yield data recorded during 1994–95 and 1996–97. The effect of IPCV-H infection on seed viability was analysed by a comparison of the proportion in independent samples for the binomial distribution (Z statistic) (Snedecor & Cochran, 1980).

Results

Symptomatology

In wheat, symptoms were first noticed 2–3 weeks after emergence. All early-infected plants were stunted and rosetted, with dark green leaves (Fig. 2a,b). These symptoms resembled those caused by the soil-borne wheat mosaic virus (SBWMV) (Wiese, 1977; Brakke & Langenberg, 1988). Chlorotic streaks (Fig. 2c) were noticed on newly emerged leaves, which subsequently became necrotic. The root system was poorly developed in early-infected plants, and most of these plants died. Plants infected later remained stunted, with dark green old leaves and young leaves showing chlorotic streaks. They produced malformed spikes, sometimes enclosed in a curled flag leaf, and fewer tillers than healthy ones. The spikes were not properly filled, and kernels from infected plants were shrivelled and dark brown. IPCV-H-infected barley plants were stunted and bushy, with chlorotic or necrotic leaves (Fig. 2e,f). Most of the

Table 2 Effect of Indian peanut clump virus (Hyderabad isolate, IPCV-H) on 3 yield components of wheat (cv. RR-21) and barley (cv. RD 103) during the 1994–95 and 1996–97 post-rainy seasons at ICRISAT-Patancheru

Crop and season[a]	Treatment[b]	Number of kernels per spike		Kernel weight per spike (g)		1000-kernel weight (g)	
		Mean[c]	Range	Mean[c]	Range	Mean[c]	Range
wheat							
1994–95	Healthy	36	33–40	1·29	1·25–1·59	36	35–42
	Infected	12	1–40	0·28	0·01–1·66	21	2–51
1996–97	Healthy	35	13–56	1·22	0·30–2·20	34	11–52
	Infected	18	1–46	0·33	0·01–1·60	17	1–47
barley							
1994–95	Healthy	36	21–46	1·64	0·70–2·20	45	21–60
	Infected	18	5–46	0·69	0·10–2·10	38	5–52

[a]For both apparently healthy ($n = 289$) and infected ($n = 351$) wheat spikes for the 1994–95 and 1996–97 seasons, respectively, and 50 barley spikes were individually analysed.
[b]When tested by ELISA, all infected plants contained the viral antigen in the flag leaf.
[c]Means within a column for one season differ significantly (rank sum test, T significant at $P < 0·001$).

infected plants died. Those that reached maturity produced poorly developed spikes.

The virus was readily detected by ELISA in roots and leaves of wheat seedlings (4 out of 90 tested) collected 2 weeks after emergence, although these plants did not show any overt symptoms. Subsequently, and until harvest, all 191 plants with symptoms tested positive, whereas 319 apparently healthy plants tested negative by ELISA. In ISEM, seven naturally infected plants were tested: typical IPCV particles from the five wheat plants and two barley plants could be trapped and were fully decorated with IPCV-H antiserum. No virus particles could be trapped from two apparently healthy plants. All the ELISA-positive plants also contained IPCV-H RNA, as tested by nucleic acid hybridization tests (Fig. 3).

P. vulgaris inoculated with leaf extracts from naturally infected wheat plants developed typical symptoms. Wheat plants, root-inoculated (with the help of an airbrush) with virus isolated from peanut and multiplied on *P. vulgaris*, showed dark green leaves and stunting, but symptoms were less severe than those observed under field conditions. All plants tested positive by ELISA.

Yield loss and seed quality

The yield components studied during the 1995–96 season are presented in Table 1. IPCV-H infection caused severe losses of wheat yield. Plant height was reduced by more than half compared with healthy plants. Infected plants produced 42% less total biomass, including 31% loss of straw and 58% loss of grain (Fig. 2d). The plant population was affected because of the death of early-infected plants. Grain was of poor quality compared with that of healthy plants and contained a larger proportion of immature kernels. The harvest index was lower for infested patches than for healthy ones. During the 1994–95 and 1996–97 seasons, IPCV-H infection severely reduced the number and weight of kernels per spike as well as the weight of 1000 kernels (Table 2). IPCV-H significantly reduced the germination of wheat and barley seed (Table 3) although wide variability from plant to plant was observed in the percentage of germination.

Table 3 Effect of IPCV-H infection on wheat (cv. RR-21) and barley (cv. RD 103) seed viability

Crop	Season	Number of seeds germinated/ number of seeds tested			Mean germination (% (range))		
		Date of test	Healthy plants[a]	Infected plants	Healthy plants[a]	Infected plants	*P*
Wheat[b]	1994–95	25/11/1996	598/600	538/600	99·7 (97–100)	89·7 (50–100)	< 0·001
Wheat	1995–96	16/05/1997	582/600	398/600	97·0 (80–100)	66·3 (0–100)	< 0·001
Barley[c]	1994–95	09/05/1997	58/100	37/86	58·0 (20–90)	43·0 (32–71)	0·041

[a]All apparently healthy plants were sampled in disease free areas and the flag leaf of all infected plants tested positive by ELISA.
[b]Seeds collected from 29 infected and 20 healthy spikes for the season 1994–95 and from 20 healthy and 35 infected spikes for the season 1995–96.
[c]Seeds collected from 5 infected and 3 healthy spikes.

Table 4 Frequency of seed transmission of IPCV-H in wheat (cv. RR-21)

Season	Date of ELISA test	Number of seeds germinated/number of seeds tested	Germination (%)	Number of seedlings tested positive	Seed transmission (%)
1994–95	11/10/1995	854/1017	84	4	0·47
	08/11/1996	787/934	84	10	1·27
	25/11/1996	1172/1247	94	4	0·34
1995–96	16/05/1997	1240/2181[a]	57	13	1·05
1996–97	06/05/1997	737/2518[a]	29	8	1·08
Total		4790/7897	61	39	0·81

[a]Kernels derived from 250 spikes.

Frequency of IPCV-H seed transmission in wheat and barley

All the seeds collected from infected wheat and barley plants, either treated with Na_3PO_4 or not, contained the viral antigen, as tested by ELISA. The frequency of seed transmission in wheat is presented in Table 4 for seed lots collected during the three consecutive post-rainy seasons. Although infection by IPCV-H resulted in poor germination, seed transmission was observed in about 1% of the seedlings. Wheat seeds stored for more than a year at 4°C still transmitted the virus. Seedlings infected through seed contained the virus in both leaves and roots. Symptoms were somewhat similar to those on inoculated plants maintained under glasshouse conditions. The seeds that transmitted IPCV-H originated from different plants, thus excluding the possibility of cross-infection during the growth of the seedlings in Petri dishes or pots. Virus presence in ELISA positive seedlings was confirmed by infectivity assays. Out of 86 barley seeds collected from infected plants, 37 germinated. None of the seedlings was found to be infected by the virus when tested by ELISA.

Surveys of wheat crops in Rajasthan

During the surveys conducted in 1994–95 and 1995–96, the virus could not be detected either in leaves, roots or seeds of cultivars Raj 3077 and Raj 1482 from a number of samples collected from three locations (558 plants tested). However, during 1996–97, when RR-21 was grown in IPCV-D-infested fields, the plants showed uniform stunting in known infested patches, without any overt symptoms. In each of the three locations, IPCV-D was detected in roots and leaves of a restricted number of RR-21 plants (3/228). Wheat plants testing negative at the time of sampling, and then maintained for a month in a glasshouse, also gave negative results by ELISA. IPCV-D could not be detected in more than 900 wheat seeds of cv. RR-21 collected from infested plots.

Polymyxa sp. detection

A few resting spores of *Polymyxa* sp. were observed in roots of a limited number of IPCV-H-infected wheat plants (3/35), but none could be detected in barley roots (0/10).

Discussion

When mechanically inoculated onto wheat, PCV caused systemic mosaic and stunting symptoms (Thouvenel & Fauquet, 1981). Using *Polymyxa*-infested soil or *Polymyxa*-infected roots as inoculum, IPCV could be transmitted to wheat under glasshouse conditions (Ratna *et al*., 1991) but the authors did not mention any symptoms. Earlier studies (Delfosse *et al*., 1995b,c) and the present study showed for the first time that infection by IPCV can cause diseases in wheat and barley crops under natural conditions. Symptoms on wheat are similar to those caused by SBWMV. However, there is no serological relationship between the two viruses (Reddy *et al*., 1985) and their genome organization differs substantially (Wesley *et al*., 1994; Miller *et al*., 1996; Naidu *et al*., 1996).

The yield reduction in wheat infected with IPCV-H was very severe and consistent over the 3-year period. Grain yield loss caused by IPCV-H infection was as high as 58% (equal to a yield reduction of 1800 kg ha^{-1}). This is similar to the wheat loss caused by severe infection by two other *Polymyxa*-transmitted viruses in North America: SBWMV in Florida, Kansas and Nebraska (Kucharek & Walker, 1974; Campbell *et al*., 1975; Palmer & Brakke, 1975; Nykaza *et al*., 1979) and wheat spindle streak mosaic virus (WSSMV) in New York and Georgia (Cunfer *et al*., 1988; Miller *et al*., 1992). It was difficult to assess the effects of SBWMV and WSSMV on yield in controlled experiments because these viruses are difficult to transmit mechanically and their vector, *P. graminis*, is mostly prevalent in low-lying, poorly drained areas of the fields where waterlogging can also affect the yield (Bays *et al*., 1985; Miller *et al*., 1991). As IPCV disease occurs mainly in well-drained, sandy soils or sandy loam soils (Delfosse *et al*., 1997), this conflict was not encountered. However, care was taken to compare plants with and without symptoms within similar soil environments.

In Andhra Pradesh, IPCV-H infection in wheat and barley caused severe symptoms and yield loss under the prevailing climatic conditions. In Rajasthan, however,

IPCV-D could not be detected in currently grown wheat cultivars. It was found only at low incidence, in wheat cv. RR-21, which showed stunting in areas of the fields known to be infested with IPCV-D. This occurred in the same patterns as that of peanut clump disease in peanut during the previous rainy season. There are thus indications that some wheat cultivars may show some resistance to IPCV-D. Yield loss caused by IPCV-D in wheat and barley crops has yet to be investigated in Rajasthan. Temperature seems to be an important factor regulating disease incidence. Winters are cooler in Rajasthan than in Hyderabad. The normal minimum, mean and maximum air temperatures are 9, 16 and 24°C in Jaipur, Rajasthan, and 15, 21 and 28°C in Hyderabad, Andhra Pradesh, in December, and 8, 15 and 22°C, and 15, 22 and 29°C, respectively, in January.

At the same time, IPCV-H incidence in the wheat crop grown in 1994–95 in Hyderabad was lower than in the crop grown during the 1995–96 and 1996–97 seasons. In 1994–95, infected plants were scattered among healthy ones, and occurred only in the areas where high disease incidence was recorded in peanut crops raised in previous rainy seasons. However, for the successive seasons, the infection was uniformly distributed and was present in areas of the field where the disease has never been observed on peanut crops. It is likely that temperature affected the infection by *Polymyxa* sp., and, consequently, the virus transmission. The 1994–95 season in Hyderabad was characterized by lower temperatures than the other two seasons. The minimum, mean and maximum air temperatures for December 1994 were 7, 19 and 29°C while for December 1995 and 1996 they were 10, 21 and 30°C and 10, 20 and 29°C, respectively. Legrève *et al*. (1998) observed that the *Polymyxa* isolated from the same experimental field of the ICRISAT-Patancheru farm had a very narrow temperature range, with an optimum between 27°C and 30°C, delayed development at 23–26°C, and almost no development at 19–22°C. The low temperatures prevalent during the 1994–95 post-rainy season at Hyderabad may not have been conducive for *Polymyxa* activation, and therefore virus transmission to wheat was low. If the *Polymyxa* occurring in Rajasthan has a similar temperature requirement to that of the Hyderabad isolate, temperatures prevalent in Rajasthan would not be conducive for fungus infection and virus transmission to winter wheat. Wheat is also grown below the tropic of Cancer in Madhya Pradesh, Maharashtra, Gujarat and Karnataka. Winters in these areas are not as cold as in Rajasthan, having temperatures similar to Andhra Pradesh. Therefore if IPCV is present in these areas it is likely that it can infect wheat crops.

Even though wheat and barley showed high incidence of IPCV-H under natural conditions they do not appear to be as good hosts of *Polymyxa* sp. as sorghum and pearl millet (Legrève *et al*., 1996). The fungus was detected only as a trace of infection on wheat plants infected under natural conditions. As in the case of peanut, wheat and barley may act as fortuitous hosts, leading *Polymyxa* to enter the resting spore stage preferentially rather than favouring fungus multiplication through secondary zoospores. These results contradict those of Ratna *et al*. (1991) and Nolt *et al*. (1988), who readily detected *Polymyxa* sp. in wheat roots from plants grown on IPCV-H-infested soil. It is presumed that the continuous use of peanut, a fortuitous host for *Polymyxa*, as a sole crop since 1987, induced a reduction in *Polymyxa* inoculum potential in the experimental field, thereby probably reducing the chance of *Polymyxa* infection to wheat and barley from resting spores.

IPCV infection affected the germination and induced variability in the germination percentage in wheat and barley. Nevertheless, wheat seedlings infected through seed grew well under glasshouse conditions and the frequency of seed transmission in wheat was close to 1%. As wheat is sown at a rate of $\approx 100\,kg\,ha^{-1}$, corresponding to $2{\cdot}5 \times 10^6$ seeds/ha, there is a high risk of spreading the virus if seed is collected from infested fields. The virus was detected in roots of seedlings infected through seed. Therefore it is most likely that isolates of *Polymyxa* that infect and multiply on wheat can acquire the virus from plants infected through seedborne inoculum. Preliminary experiments have shown that nonviruliferous *Polymyxa* could acquire the virus from wheat and maize plants infected by IPCV-H through seed-borne inoculum, and transmit the virus to plants grown in an automatic immersion tank system (Delfosse & Legrève, unpublished). IPCV has a wide temperature range and mechanical inoculation onto wheat results in infection at temperatures between 15 and 30°C (Reddy *et al*., 1988). Thus, there is a potential risk to wheat if IPCV is established in temperate areas where *Polymyxa* sp. is adapted to wheat and to low temperatures.

Few rod-shaped, *Polymyxa*-transmitted viruses were reported to be transmitted through seed. These include: *Nicotiana veluntina* mosaic, a proposed furovirus (Randles, 1978); potato mop top virus, which is vegetatively transmitted through seed tubers; and PCV and IPCV (Mink, 1993). To our knowledge, seed transmission of SBWMV has been investigated for low temperature strains that are usually no longer detected in aerial parts of plants when the temperature rises in late spring and summer. The virus was reported not to be seed transmitted (Brakke, 1971). However, Wiese (1977) mentioned that SBWMV sometimes spreads more rapidly and over larger distances than can be explained by soil movement. Rubies-Autonell & Vallega (1991), reported the presence, in the region of Rome, Italy, of SBWMV particles in wheat leaves analysed by ISEM, well beyond the heading stage. They could even detect them in immature seed of cv. Valnova. More recently a Polish isolate of SBWMV was found to be seed-transmitted in rye, and seedborne, but not seed-transmitted, in wheat (Jezewska, 1995). Considering that IPCV is seed-transmitted in wheat, three millets (Reddy *et al*., 1998), and maize (Delfosse *et al*.,

unpublished), and that high-temperature strains of SBWMV exist, it is suggested that the seed transmission of *Furovirus* be reinvestigated.

Polymyxa sp. is a ubiquitous fungus. Care should be taken in germplasm movement to avoid the spread of furoviruses and pecluviruses. The risk of IPCV acquisition by several temperate strains of *Polymyxa* sp. is currently being investigated.

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