

Survey of Chickpea (*Cicer arietinum* L.) for Chickpea Stunt Disease and Associated Viruses in India and Pakistan

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ABSTRACT

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Chickpea chlorotic dwarf geminivirus (CCDV) and some luteoviruses were associated with chickpea stunt disease in India and Pakistan. One thousand eight hundred and four plants with stunt disease symptoms were collected and tested with poly- and monoclonal antibodies. Bean leafroll luteovirus (BLRV)-like luteoviruses and viruses reacting with an antiserum to a luteovirus isolate from chickpea, tentatively referred to as chickpea luteovirus (CpLV), were involved. Relative prevalence of the viruses varied among the different chickpea-growing areas. The BLRV-like viruses were of minor importance, while CCDV and CpLV-like viruses were widely distributed. The reaction patterns of the luteoviruses from chickpea with monoclonal antibodies differed from those of some known luteoviruses. In addition to CpLV, BLRV, and other luteoviruses, an unidentified, graft-transmissible agent may be involved in the etiology, which is more complex than reported initially.

India is the largest chickpea producer in the world, growing 4 million tons annually on 6.5 million ha. Pakistan, ranking second, produces 0.5 million tons annually on 1 million ha. Despite the high total production, yields of chickpea are low due to many constraints. In order of importance, drought and fungal and viral diseases are major limiting factors in chickpea production (21).

Chickpea stunt is the most important virus disease of chickpea. It is characterized by leaf reddening in desi-type and yellowing in kabuli-type chickpeas. Internode shortening, plant stunting, and phloem browning in the collar region are observed in both types (17). These symptoms have also been reported from many other chickpea-growing areas in India and other countries (4,5,10,12,20). Plant decline, ranging from poor performance to premature death of diseased plants, can dramatically reduce production. Kaiser and Danesh (13) reported 90 to 100% yield losses when chickpea plants were inoculated with bean leafroll luteovirus (BLRV), reported to cause chickpea stunt (19). Kotasthane and Gupta (15) found 80

to 95% yield losses in chickpea by chickpea stunt. Chickpea chlorotic dwarf geminivirus (CCDV), also provoking symptoms characteristic of chickpea stunt (9), caused 75 to 100% yield losses depending on the time of infection (8).

Chickpea stunt in India has been ascribed to BLRV (19), although the identity of the virus, first isolated and characterized in the Netherlands (2), has not been established. In California, other luteoviruses, namely subterranean clover red leaf virus (SCRLV, a strain of soybean dwarf virus), legume yellows virus (LYV, probably a strain of BLRV), and beet western yellows virus (BWYV), have been shown to infect chickpea and to cause symptoms similar to those of chickpea stunt (4). BWYV and BLRV were also reported to infect chickpea in Spain (5). In India, leafhopper-transmitted CCDV was recently found to incite symptoms in chickpea similar to those described for chickpea stunt (9). Thus, it appears that a geminivirus and a number of luteoviruses can cause similar, if not identical, symptoms in chickpea.

No data are available on the viruses actually involved in chickpea stunt disease in farmers' fields or on their relative importance. Such information is essential for developing control strategies. Therefore, surveys were conducted to identify viruses associated with chickpea stunt and to assess their relative incidence. This paper reports the results of these surveys in India and Pakistan during the 1991 to 1992 season. Results are also presented on the pre-

liminary characterization of newly detected luteoviruses by poly- and monoclonal antibodies.

MATERIALS AND METHODS

Areas surveyed. In India, surveys were conducted in the states of Rajasthan, Madhya Pradesh, and Gujarat (Fig. 1) during January and February 1992. Chickpea fields were chosen with the assistance of researchers familiar with chickpea production in these areas. Crops raised at research stations in the areas surveyed and at Anand (Gujarat), Hisar (Haryana), and Patancheru (the ICRISAT Asia Center, Andhra Pradesh), were also included (Fig. 1). In Pakistan, chickpea-growing areas in Punjab (Fig. 1) were visited during February 1992, the main area being the Thal, where 70% of Pakistan's chickpea production takes place (14). The inspected fields were chosen systematically by making a stop after every 5 km during the trips, or at the nearest chickpea field thereafter.

Observations and sample collection. At each field visited, the size of the field, stage of crop development, cropping pattern, crop density, and stunt incidence were recorded. The incidence of stunt was assessed by counting the number of plants with stunt symptoms in five randomly distributed groups of 100 plants each. Whenever possible, samples were collected for further testing from 10 to 15 plants with characteristic symptoms in each field (Fig. 2). Samples collected in India and Pakistan were processed at the ICRISAT Asia Center, Patancheru, and the National Agricultural Research Centre (NARC), Islamabad, respectively.

Serology. All plant samples were tested with polyclonal antisera in double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA), as described by Clark and Adams (6). Polyclonal antiserum to BLRV was used since this virus had been the only luteovirus reported from chickpea in India. In preliminary tests, a luteovirus that did not react with BLRV polyclonal antiserum was found in many chickpea plants with symptoms of chickpea stunt at the ICRISAT Asia Center. Luteovirus-like particles were observed with the electron microscope. This virus was purified and a polyclonal antiserum was produced. In reciprocal DAS-ELISA,

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this antiserum did not react with the type isolate of BLRV (2), and BLRV antiserum did not react with the isolate from chickpea (data not shown). This isolate is thus serologically distinct from BLRV, and was referred to as chickpea luteovirus (CpLV).

The samples collected during the surveys were initially screened using polyclonal antisera to BLRV, CpLV, and CCDV. In those cases in which hardly any of the collected samples in an area reacted with the antisera used, a number of the samples were also tested against polyclonal antisera to potato leafroll luteovirus (PLRV) and SCRLV.

The samples, consisting of leaf and stem tissue, were ground in 0.02 M phosphate-buffered saline containing 0.05% Tween 20 (PBS-T) and 2% polyvinylpyrrolidone (20 ml of buffer per gram of plant material). Samples reacting with one of the luteovirus polyclonal antisera were tested with the monoclonal antibodies (Mabs) in a triple antibody sandwich-ELISA. Coating was done with BLRV or CpLV polyclonal antibodies at a concentration of 2 µg/ml, and plant extracts prepared in PBS-T were used. The Mabs were used in the concentrations mentioned below, and a goat-anti-mouse alkaline phosphatase conjugate was used at a 1:1,000 dilution.

The antiserum to BLRV (2) was supplied by L. Bos (the Netherlands), to PLRV by D. Z. Maat (the Netherlands), and to SCRLV by G. R. Johnstone (Australia; 11). The antiserum to CCDV (9) and to CpLV had been produced at the ICRISAT Asia Center. The Mabs to PLRV had been produced at the Wageningen Agricultural University (WAU), the Netherlands. The Mabs used in this study, because of their differential reaction to a number of well-described luteoviruses (23), and their dilutions (in parentheses), were WAU-A2 (1,000x), WAU-A6 (5,000x), WAU-A7 (5,000x), WAU-A12 (2,000x), WAU-A13 (1,000x), WAU-A24 (2,000x), WAU-A47 (2,500x), and WAU-B9 (1,000x). In addition, a Mab to barley yellow dwarf luteovirus (BYDV), IL-1 (1,000x) (7), was also used.

Purification, electron microscopy and transmission. The purification procedure as described by Horn et al. (9) was applied up to the sucrose gradient. The partially purified samples were then observed, after staining with 1% uranyl acetate, with a Philips 201 C electron microscope. Sap transmission was done by triturating chickpea leaflets in 50 mM potassium phosphate buffer (pH 7.0). The extract was then used to manually inoculate Carborundum-dusted leaves of chickpea plants in the greenhouse. Aphid transmission was done using *Aphis craccivora* Koch and *Myzus persicae* Sulzer. The aphids were allowed to feed for 1 day on stems and leaflets of chickpea plants collected from the field. They were then transferred to healthy chickpea plants in the greenhouse

and allowed to feed on them for 4 days. Tips of field-collected chickpea plants were grafted on healthy chickpea plants in the greenhouse to study graft transmission.

RESULTS

In the areas surveyed, 90 farmers' fields (57 in India, 33 in Pakistan) and 10 re-

search stations were visited. In total, 1,804 chickpea plants (1,600 from India, 204 from Pakistan), showing some or all of the symptoms characteristic of stunt, were collected and tested in ELISA.

Survey in India. Ten experimental chickpea fields at the ICRISAT Asia Center were surveyed during the season. The



Fig. 1. The chickpea-growing areas surveyed for chickpea stunt in India and Pakistan during the 1991 to 1992 growing season.



Fig. 2. Field symptoms of chickpea stunt. Infected plant, on the right, surrounded by healthy chickpea plants.

incidence of stunt was always less than 1%. Of 699 plants tested in ELISA, 396 reacted with CCDV antiserum, 36 with CpLV antiserum, and two with BLRV antiserum. None of the samples reacted with antisera to SCRLV or PLRV. Most CpLV-positive plants were from three fields.

In Gujarat, 14 farmers' fields (of 0.2 to 3.0 ha), near Junagadh, and experimental plots at the Junagadh and Anand Agricultural Research Stations of the Gujarat Agricultural University were visited. Here, the crop was at the pod-setting and filling stage. Stunt incidence in farmers' fields ranged from 0 to 45% (average 12%). Only eight (all from farmers' fields) of the 217 samples tested (167 from 14 farmers' fields, 33 from Junagadh, and 17 from Anand) reacted with CCDV antiserum, and 106 (79 from farmers' fields, 11 from Junagadh, and 16 from Anand) reacted weakly with CpLV antiserum. No CCDV was found at either research station, but a high proportion of the samples from these stations reacted (weakly) with CpLV antiserum. Six samples from Anand reacted with BLRV antiserum.

In Haryana, one experimental chickpea field, at the Government Livestock Farm at Hisar, was surveyed before flowering (December 1991) and during flowering (February 1992), but different plants were sampled each time. Of the 308 plants tested, 114 reacted with CCDV antiserum, 8 with CpLV antiserum, and none with BLRV antiserum. No CpLV was detected in plants collected in February.

In Madhya Pradesh, 16 farmers' fields, varying in size from 0.1 to 3.0 ha, and research plots at the Khargone Agricultural Research Station were visited. The crop was at the flowering or at the pod-setting stage. The incidence of stunt ranged from 0 to 29%, the average being 4%. At the research station, incidence was 15%. Only eight (five from 16 farmers' fields and three from Khargone Station), of the 210 samples collected, reacted with CCDV antiserum, and none reacted with the four luteovirus antisera used. A number of plants that did not react with any of the antisera used were subjected to other tests including purification, sap and aphid transmission, or grafting. The suspension obtained after submitting plants to the purification protocol was observed with the electron microscope and no virus particles were observed. Sap and aphid transmissions were also unsuccessful, but stunt-like symptoms could be reproduced by graft transmission (data not shown).

In Rajasthan, 27 farmers' fields, varying in size from 0.25 to 2.5 ha, and research plots at Durgapura Agricultural Research Station and Diggi Agricultural Research Substation of the Rajasthan Agricultural University were visited when the crop was at early flowering. Stunt incidence was low (0 to 5.2%). In six fields not a single infected plant was found. In three fields stunt incidence, only due to CCDV, ranged from 2.6 to 5.2%. At the two research stations visited, stunt incidence was <0.1% and all 47 plants collected there were in-

fectured with CCDV. Plants infected with CpLV-like (five) or BLRV-like (two) viruses were found only in a few fields in Rajasthan.

Survey in Pakistan. In the Thal area (Punjab), 28 farmers' fields were surveyed. The crop was at the flowering stage. Stunt incidence was generally low (0 to 2.6%). Of the 148 samples collected from farmers' fields, 74 reacted with CCDV antiserum, 9 with CpLV antiserum, and none with BLRV antiserum. Two fields were visited at a research station in Kallurkot in the western part of the Thal area. Ten of 15 samples there were positive for CCDV and two for CpLV. The CCDV incidence was low in local cultivars, whereas in exotic germ plasm it was up to 12%.

In the Attock and Chakwal districts (Punjab), five farmers' fields (three and two, respectively) and two research stations (one in each district) were visited. In the farmers' fields only CCDV was found (five of 12 samples from Attock and three of five from Chakwal), whereas at the two research stations CpLV-like viruses were detected (five at Attock and 10 at Chakwal).

Testing with Mabs. From the above-mentioned areas, 38 representative samples of those that reacted with a polyclonal luteovirus-specific antiserum (CpLV or BLRV), were selected and tested further with Mabs. Based on the reaction of the samples with the polyclonal antisera, two groups, BLRV- and CpLV-like viruses, could be distinguished. The samples that

Table 1. Reaction of selected chickpea samples from India and Pakistan with polyclonal antisera and monoclonal antibodies, as compared with the reaction of described luteoviruses as reported in the literature (7,23)

Origin of samples	Polyclonal antisera ^a	Monoclonal antibodies									Number of samples
		PLRV WAU ^b						BYDV ^c			
		A 2	A 6	A 7	A 12	A 13	A 24	A 47	B9	IL1	
CpLV-like viruses											
ICRISAT	CpLV				S ^d	-	M		S	-	3
	CpLV				S	-	M		W	-	3
	CpLV				S	-	W		M	-	1
Rajasthan	CpLV				S	-	-		-	-	5
Hisar	CpLV				S	-	M		M	-	3
Pakistan	CpLV				S	-	M		W	-	6
Junagadh	CpLV				S	-	S		W	-	7
BLRV-like viruses											
ICRISAT	BLRV				-	-	W		W	S	
Rajasthan	BLRV				W	S	-		-	S	
Anand	CpLV				S	-	S		M	S	
Described luteoviruses											
	BLRV				-	-	S				
	BWYV ^e				S	W	M				
	BMVY ^f				S	S	M				
	PLRV				S	S	S				

^a Chickpea luteovirus (CpLV) and bean leafroll luteovirus (BLRV). Polyclonal antiserum with which the samples reacted in double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA). This antiserum was used in triple antibody sandwich-ELISA for coating.

^b Potato leafroll virus. Wageningen Agricultural University monoclonal antibodies.

^c Barley yellow dwarf luteovirus.

^d Reactions in ELISA: S = strong (OD > 0.6), M = medium (0.6 > OD > 0.3), W = weak (0.3 > OD > 0.1), - = OD < 0.1 at room temperature, 1 to 2 h after addition of substrate. OD = optical density.

^e Beet western yellows virus.

^f Beet mild yellows virus.

reacted with CpLV polyclonal antiserum did not react with BLRV polyclonal antiserum, and had been collected from the ICRISAT Asia Center, Rajasthan, Hisar, and Pakistan. These samples reacted with one or more of the Mabs WAU-A12, WAU-A24, and WAU-B9 and not with the other Mabs used (Table 1). The samples from Rajasthan differed in that they reacted only with WAU-A12. Those from Junagadh reacted weakly with CpLV polyclonal antiserum. They also reacted with WAU-A12, WAU-A24, and WAU-B9, but their reaction with WAU-A24 was stronger than that of the other CpLV-like viruses.

Samples that reacted only with BLRV polyclonal antiserum had been collected at the ICRISAT Asia Center and in Rajasthan. These samples all reacted strongly with the BYDV Mab IL-1, and their reaction with the PLRV Mabs was different in spectrum and intensity for the two areas. In only one case, from Anand, were samples positive for both BLRV and CpLV polyclonal antisera. Whether a double infection with a BLRV-like and a CpLV-like virus occurred or one luteovirus was reactive with both polyclonal antisera is unknown.

DISCUSSION

Survey data for the viruses involved in chickpea stunt failed to resolve the etiology of the disease. At different places different viruses, causing identical symptoms, were found to be involved or to predominate (Table 2). A number of luteoviruses dissimilar to any of the known legume luteoviruses were detected. Tentative characterization of the new luteoviruses provided information that will facilitate future surveying.

Of the 1,804 samples tested, 42% reacted with CCDV antiserum, 10% with CpLV antiserum, and 0.6% with BLRV antiserum. CCDV was the predominant virus at the locations surveyed in Rajasthan, at Hisar, and at the ICRISAT Asia Center, in India, and in Pakistan. With respect to the luteoviruses, the results reported here provide evidence that BLRV is not the only virus of this group involved in chickpea stunt. Of all samples containing a luteovirus and reacting with antiserum to either CpLV or BLRV, very few reacted with both. This corroborates the discrimination of CpLV as a serologically distinct luteovirus. The CpLV-like viruses appeared to be more widely distributed in India and Pakistan than the BLRV-like viruses. The latter were only found at two locations in India and their incidence was low. Thus, at least two distinct luteoviruses, viz., a BLRV-like and a CpLV-like virus, were present. Another luteovirus or luteovirus strain may have been involved in Gujarat. The reaction patterns of all isolates tested with the Mabs are different from those of known luteoviruses (Table 1). Thus, new luteoviruses may well have

been detected here. Further tests, including host range and vector specificity, are needed to identify the luteoviruses as distinct viruses or strains, especially to explain the variation within the BLRV-like viruses.

Forty-seven percent of the samples with stunt symptoms did not react with any of the antisera to luteoviruses used here and with CCDV antiserum. For example, only 8 of the 210 samples collected in Madhya Pradesh reacted with antiserum to CCDV, and none with antisera to four luteoviruses. Grafting, however, indicated that a transmissible agent is involved. The stunt symptoms are not characteristic of any of the other viruses known to infect chickpea, e.g., alfalfa mosaic virus, cucumber mosaic virus, potyviruses (1,16,18). Also nonviral factors could have caused some of the symptoms characteristic of chickpea stunt. Leaf reddening may be induced by several types of stress, either biotic or abiotic (3,17).

Low incidences of stunt associated with CCDV in local cultivars (land races) and relatively high stunt incidences associated with CCDV in introduced genotypes at the research station in Kallurkot (Pakistan) indicate a potential threat from CCDV. New genotypes should be screened for resistance to CCDV prior to introduction into areas where CCDV is endemic. Low stunt incidence in local genotypes suggests that they have already been selected for resistance.

Some observations on the epidemiology of luteoviruses were made during the surveys. The occurrence of these viruses in a few fields suggests that their spread was limited or that their sources of infection were localized. Other observations (data not shown) indicated effects of cropping pattern on the incidence of luteoviruses. They seemed to occur most at sub-optimal plant densities, and more in monocultures than in mixed cropping systems. Such epidemiological aspects may be important for disease control (3,22), and they require more extensive study.

The CCDV and CpLV-like viruses pose a threat to chickpea production. Future

surveys done at different times during the chickpea-growing season should monitor incidence and shifts in virus occurrence. The luteoviruses occurring in chickpea are now being further characterized and assessed for their importance. Furthermore, weed species in chickpea fields were found to harbor a luteo- and/or a geminivirus, and can therefore serve as reservoir host. The identity of these weed species and their role in the ecology of these viruses is still under study.

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Table 2. Summary of the results of surveys for chickpea stunt disease during the 1991 to 1992 season

Country/area	Stunt incidence	Predominant virus ^a	Minor virus(es) ^a
India			
ICRISAT (Andhra Pradesh)	Low	CCDV	CpLV-like BLRV-like
Gujarat (Junagadh)	High	A luteovirus ^b	CCDV
Gujarat (Anand)	High	A luteovirus ^b	None
Hisar (Haryana)	Low-High ^c	CCDV	CpLV-like
Madhya Pradesh	High	Unknown agent	CCDV
Rajasthan	Low	CCDV	CpLV-like BLRV-like
Pakistan	Low	CCDV	CpLV-like

^a CCDV = chickpea chlorotic dwarf geminivirus; BLRV = bean leafroll luteovirus; CpLV = chickpea luteovirus.

^b Weak reaction with CpLV antiserum.

^c Stunt incidence high in stunt nursery ICRISAT, low in other fields.

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