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Diversity among the coat proteins of luteoviruses associated with chickpea stunt disease in India†

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Summary

Chickpea stunt is an important virus disease of chickpea in the Indian subcontinent which is thought to be caused by infection with a luteovirus. Samples of diseased chickpea plants were collected from different chickpea growing regions of India and analysed with a panel of monoclonal antibodies to potato leafroll, beet western yellows and barley yellow dwarf (RPV strain) luteoviruses. The results suggested that more than one luteovirus was present in chickpea crops near ICRISAT Asia Center, Hyderabad. Aphid transmission tests resulted in the separation of two distinct isolates from these samples. One of them (isolate L) was more efficiently transmitted by *Myzus persicae* than the other (isolate IC). Nucleotide sequence analysis of DNA obtained by reverse transcription-polymerase chain reaction (RT/PCR) amplification revealed that the amino acid sequence of the coat protein of isolate L was 94% identical to that of beet western yellows virus, whereas the coat protein sequence of isolate IC was 82% identical to that of isolate L and 80% identical or less to those of the coat protein of other luteoviruses. Using newly designed "universal luteovirus primers", a minor sequence variant of isolate IC, which was 96% identical to it in part of the coat protein gene, was detected in the same location during the next season. Only isolate IC could be detected in samples from other locations by either serological or nucleotide sequence analysis.

Key words: Chickpea stunt disease, luteovirus, monoclonal antibodies, coat protein, RT-PCR, universal luteovirus PCR primers

Introduction

Chickpea (*Cicer arietinum*) is an important high-protein pulse crop cultivated in four eco-geographic regions, namely the Indian Subcontinent, West Asia and North Africa, East Africa and the Americas. Chickpea stunt is a serious virus disease prevalent in these chickpea-growing regions. The characteristic symptoms of the disease are stunting due to shortening of internodes, leaf reddening (in the case of desi types), or yellowing (in the case of kabuli types), and browning of the phloem in the collar region (Nene & Reddy, 1987; Nene *et al.*,

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1991). Stunting is most conspicuous when plants are infected at an early stage of growth and many such plants die prematurely. In plants infected when they are more mature, stunting may not be obvious, but plant discolouration and phloem browning are usually evident. In India, chickpea stunt disease has been attributed to infection by bean leaf roll luteovirus (BLRV: Reddy, Nene & Verma, 1979). Pea leafroll luteovirus (synonymous with BLRV: Ashby, 1984) has also been reported to infect chickpea in Iran (Kaiser & Danesh, 1971). In addition, chickpea crops have been reported to be infected by subterranean clover red leaf, a strain of soybean dwarf (SDV: Randles & Rathjen, 1995), beet western yellows (BWYV) and legumes yellows (a strain of BLRV: Randles & Rathjen, 1995) luteoviruses in California (Bosque-Perez & Buddenhagen, 1990), and BLRV and BWYV in Spain (Carazo *et al.*, 1993). In all these instances, diagnoses were made on the basis of results of serological assays.

When disease surveys were made of chickpea plants with stunt disease collected from different parts of India using a panel of monoclonal antibodies (MAbs) to potato leafroll luteovirus (PLRV) and barley yellow dwarf luteovirus (BYDV-RPV), a number of luteoviruses were detected (Horn, Reddy, van den Heuvel & Reddy, 1996). However, in that work it was not clear if this situation was due to the presence of distinct strains of a particular luteovirus, assumed to be BLRV, or of different luteoviruses. The presence of several luteoviruses complicates both the development of control measures and the testing for sources of resistance to stunt disease in chickpea germplasm.

In the present study, serology and sequence analysis of the putative coat protein gene were used to understand the apparent diversity in luteoviruses associated with stunt disease. A comparison of the coat protein gene sequences with those of well characterised luteoviruses revealed the presence of two distinct luteovirus sequences, which suggested that two different viruses were present, a strain of BWYV and a luteovirus distinct from those for which the coat protein sequence is known.

Materials and Methods

Source of plants

Chickpea plants showing stunt disease symptoms were collected in India during the 1992–93 and 1993–94 growing seasons. They were obtained from experimental fields at ICRISAT Asia Center (IAC), Hyderabad in Andhra Pradesh state and from farmers' fields in the Junagadh region in Gujarat state, the Khargone region in Madhya Pradesh state, and the Akola region in Maharastra state.

Serology

Double-antibody sandwich (DAS) and triple-antibody sandwich (TAS) forms of enzyme-linked immunosorbent assay (ELISA) were used to test samples essentially as described by Torrance (1992). Plant samples were extracted in 0.01 M phosphate-buffered saline containing 0.01 M DIECA (purified virions were diluted in the same buffer without DIECA) and added to microtitre plates (NUNC Immunoplate II) pre-coated with immunoglobulin (1 μg ml^{-1}) purified from rabbit polyclonal antibodies to chickpea chlorotic dwarf geminivirus (CCDV: Horn, Reddy, Roberts & Reddy, 1993), or to a luteovirus preparation purified from stunt-affected chickpeas (Anon., 1991). Samples of the same immunoglobulins, conjugated with alkaline phosphatase, were used in DAS-ELISA. In TAS-ELISA, MAbs to PLRV isolates from Scotland (SCR-3, SCR-6, SCR-8, SCR-10; Massalski & Harrison, 1987), The Netherlands (WAU-A12, WAU-A24 and WAU-B9; Van den Heuvel, de Blank, Goldbach

& Peters, 1990) and the Andean region (SCR-118, SCR-122 and SCR-125; L Torrance and V Flores, unpublished data), to groundnut rosette assistor virus (GRAV; SCR-111, SCR-112; Scott *et al.*, 1996), to BWYV (510-H; a gift from P Ellis, AgCanada Research Station, Vancouver) or to BYDV-RPV (IL-1; D'Arcy, Torrance & Martin, 1989) were used as the second antibody and were followed by rabbit anti-mouse IgG (whole molecule)-alkaline phosphatase conjugate. The enzyme substrate was *p*-nitrophenyl phosphate and the A_{405} were measured in a Titertek Multiscan Plus Photometer (ICN Flow) after incubation for 1 to 2 h at 20–25°C or 16 h at 4°C. A reading of A_{405} more than twice the mean of the A_{405} estimated for virus-free samples was taken to be positive.

Aphid transmission

Virus particles were purified from infected chickpea plants collected from the field as described by Horn *et al.* (1993). Electron microscopy showed that the particles were similar to those of luteoviruses and that no other types of particle could be detected. *Myzus persicae* and *Aphis craccivora* were fed on preparations of purified virions through membranes for 24 h and then allowed to feed on healthy chickpea and pea seedlings for 48 h. Subsequent transmission tests were done using *M. persicae* and pea seedlings as a host. Aphids were fed during an acquisition access period of 24 h and transferred (five aphids/plant) onto healthy pea seedlings for an inoculation access period of 48 h. Three weeks after inoculation, individual plants were assayed for virus infection by TAS-ELISA.

Extraction of nucleic acids

About 500 mg of plant tissue were ground into fine powder in liquid nitrogen and mixed thoroughly with 500 μl of 100 mM Tris-HCl (pH 8) buffer containing 2 mM EDTA and 20 g litre^{-1} SDS and 500 μl of phenol + chloroform (1/1, v/v). Extracts were incubated at 70°C for 5 min followed by centrifugation at room temperature. The aqueous phases were extracted two more times with phenol-chloroform mixture and nucleic acids were precipitated from 70% ethanol overnight at −20°C. Virus RNA was extracted from preparations of purified virus particles as described by Mayo *et al.* (1982).

Oligonucleotides

Three sets of oligonucleotide primers were used. Set 1 was essentially the "universal luteovirus primers" designed by Robertson, French & Stewart (1991), i.e. 5′-tagcatGC-CAGTGGTTA/GTGGTC (#210), which corresponds to sequence starting from nucleotide position 78 of the coat protein gene (Fig. 1) with extra sequence (lower case) added to create a *Sph* I site, and 5′-gcctcGAGTCTACCTATTTGG (#211), which is complementary to sequence commencing five nucleotides upstream of the termination codon of the coat protein gene with extra nucleotides (shown in lower case) added to create a *Xho* I site. Set 2 was 5′-gatggtcgacATGAGTACGGTCGT (#257), which corresponds to sequence commencing at the initiation codon of the coat protein gene of PLRV with extra sequence added to create a *Sal* I site (shown in lower case), and primer #211. Set 3 was primers designed to match sequence in all the luteovirus coat protein genes currently in the databases (Mayo & Ziegler-Graff, 1996). Primer #1005 was 5′-CTC/TAAGG/TCCTACCA, which corresponds to sequence starting at nucleotide 292 of the coat protein gene (Fig. 1), and primer #1004 was 5′-C/AATCTACCTATTT, which is complementary to sequence starting three nucleotides upstream of the termination codon.

RT/PCR amplification

Nucleic acids extracted from plant samples (3–5 μg) or virus RNA (0.1 μg) and 400 ng of the downstream primer (#211 or #1004) in 10 μl water were heated at 65°C for 2 min and slowly cooled to 42°C. First strand cDNA was synthesised using MMuLV reverse transcriptase (Boehringer Mannheim) according to the manufacturer's specifications. The polymerase chain reaction (PCR) mixture contained 2 μl of the first strand cDNA reaction mixture, 1 × PCR buffer (Boehringer Mannheim), 200 μM of each dNTP, 400 ng each of upstream and downstream primers and 0.5 U Taq polymerase (Boehringer Mannheim) in a total volume of 50 μl. The mixture was overlaid with 50 μl mineral oil, heated at 94°C for 5 min and then mixed with the enzyme. The reactions were cycled 35 times, each cycle comprising 90 s at 94°C, 60 s at 55°C (for upstream primers #210 and #257) or 45°C (for upstream primer #1005) and 2 min at 72°C. At the end of the cycling there was a final extension step for 5 min at 72°C.

Cloning and sequence analysis

PCR products were separated by electrophoresis in 5% acrylamide gels and the DNA was eluted from selected bands. The eluted DNA was digested with appropriate restriction enzymes and ligated into similarly digested M13 mp18 and mp19 vectors or cloned directly into pGEM-T (Promega). Sequencing was done by the dideoxy chain termination method (Sanger, Nicklen & Coulson, 1977) using the Sequenase 2.0 kit (Amersham).

Sequences were analysed using programs in the GCG package (Devereux, Haeberli & Smithies, 1984) and CLUSTALV (Higgins, Bleasby & Fuchs, 1992). Putative coat protein amino acid sequences of the viruses in the chickpea samples were compared with those of the luteoviruses BLRV (Prill, Maiss, Katul & Casper, 1990), BWYV (Veidt *et al.*, 1988; de Miranda *et al.*, 1995), PLRV (Mayo, Robinson, Jolly & Hyman, 1989), BYDV-MAV (Ueng *et al.*, 1992), BYDV-RPV (Vincent, Lister & Larkins, 1991), beet mild yellows (BMYV, Guilley, Richards & Jonard, 1995; de Miranda *et al.*, 1995), cucurbit aphid-borne yellows (CABYV, Guilley *et al.*, 1994), GRAV (Scott *et al.*, 1996), sweet potato leaf speckling (SPLSV, Fuentes *et al.*, 1996) and soybean dwarf (SDV, Rathjen *et al.*, 1994).

Results

Aphid transmission

Attempts to feed aphids directly on infected field-collected chickpea plants (obtained during the 1992–93 season at IAC) for virus acquisition were not successful. Therefore, infected material was pooled and virus was purified from it and fed to aphids through membranes. In repeated tests, *M. persicae* was able to transmit the virus but *A. craccivora* was not. When the cultures established by aphid transmission were assayed by ELISA using MAbs, two patterns of reaction were detected. The two cultures showing each pattern were designated as IC and L respectively (Table 1). The pattern of reactions was similar to that of purified virus preparation originally used for aphid transmission suggesting that the preparation had contained a mixture of two isolates. The results of limited tests suggest that the IC and L isolates differed in their transmissibility by *M. persicae*. In general, *M. persicae* was able to acquire isolate L and transmit it readily; usually all attempts to transmit it using five aphids/plant were successful. In contrast, in similar tests, *M. persicae* transmitted isolate IC only poorly (less than 10% of transmission attempts were successful). In similar assays, purified virus preparations from chickpea plants collected from IAC during 1993–

Table 1. *Reactions of luteovirus isolates from infected chickpea plants with a panel of monoclonal antibodies (MAbs)*[a]

Isolate BWYV	Year	MAbs to PLRV A12	PLRV A24	PLRV B9	BYDV-RPV RPV IL-1	BYDV-RPV 510-H
IAC[1]	1992–93	2.16	1.57	0.06	0.55	1.45
	1993–94	2.09	1.63	0.72	0.04	1.55
Junagadh[1]	1992–93	2.04	1.44	0.05	0.05	1.61
Khargone[1]	1992–93	1.93	1.56	0.07	0.05	nt
	1993–94	1.88	1.45	0.07	0.06	nt
Akola[1]	1993–94	2.04	1.63	0.05	0.06	nt
IC[2, 3]		2.13	1.52	0.06	0.04	1.86
L[2]		1.94	0.06	0.06	1.39	1.58

[a] A_{405} values are the mean of three replicate wells measured after 16 h at 4°C. Values for uninfected samples were 0.06, those for the buffer blank were 0.02.
[1] Location of the field from which the samples were taken.
[2] Cultures established by aphid transmission.
[3] The IC isolate also reacted with PLRV MAbs: SCR-111, SCR-112, SCR-118, SCR-122 and SCR-125 but not with MAbs SCR-3, SCR-6. SCR-8, or SCR-10. No tests were done with isolate L.
nt — signifies not tested.

1994, and from Junagadh, Khargone or Akola could be transmitted only poorly by *M. persicae* (< 10% efficiency). These isolates therefore resemble isolate IC in being poorly transmissible by *M. persicae*.

Serological tests

Previous studies (Horn *et al.*, 1993, 1996) have shown that chickpea plants infected with either CCDV or an unidentified luteovirus show similar, though not identical, symptoms. Therefore, diseased plants collected from different regions in India were tested for both viruses by DAS-ELISA. Plants from each region which reacted with antiserum to the luteovirus, but not with antiserum to CCDV, were then pooled. Virus preparations were purified from these plants and were used for serological assays, and for nucleic acid extraction for use in cloning experiments. Serological assays were done using a panel of MAbs to PLRV, BWYV and BYDV-RPV. The results are shown in Table 1.

Samples from all locations reacted strongly with MAb A12, A24 and 510-H (samples from Khargone and Akola were not tested with 510-H) but differed from each other in their reactivity with other MAbs; the IAC material collected in 1992–93 gave a positive reaction with IL-1 whereas material collected in 1993–94 gave a positive reaction with B9. Material from Junagadh, Khargone and Akola did not react with either IL-1 or B9. Aphid transmission studies with *M. persicae* (described above) and nucleotide sequence data (see below) suggest that (i) reaction with A24 and IL-1 in the case of 1992–93 material was due to the presence of a mixture of two distinct isolates (i.e. IC and L), (ii) reaction with A24 and B9 in the case of 1993–94 material was due to the presence of a mixture of IC type isolate and its variant, and (iii) reaction with A24 in the case of Junagadh material was due to the presence of IC type isolate alone. Although no attempt was made to estimate the relative proportion of each isolate in a mixture, the O.D values obtained using MAbs A24, IL-1 and B9 suggest that IC was more abundant than either isolate L (in 1992–93 material) or a variant of IC (in 1993–94

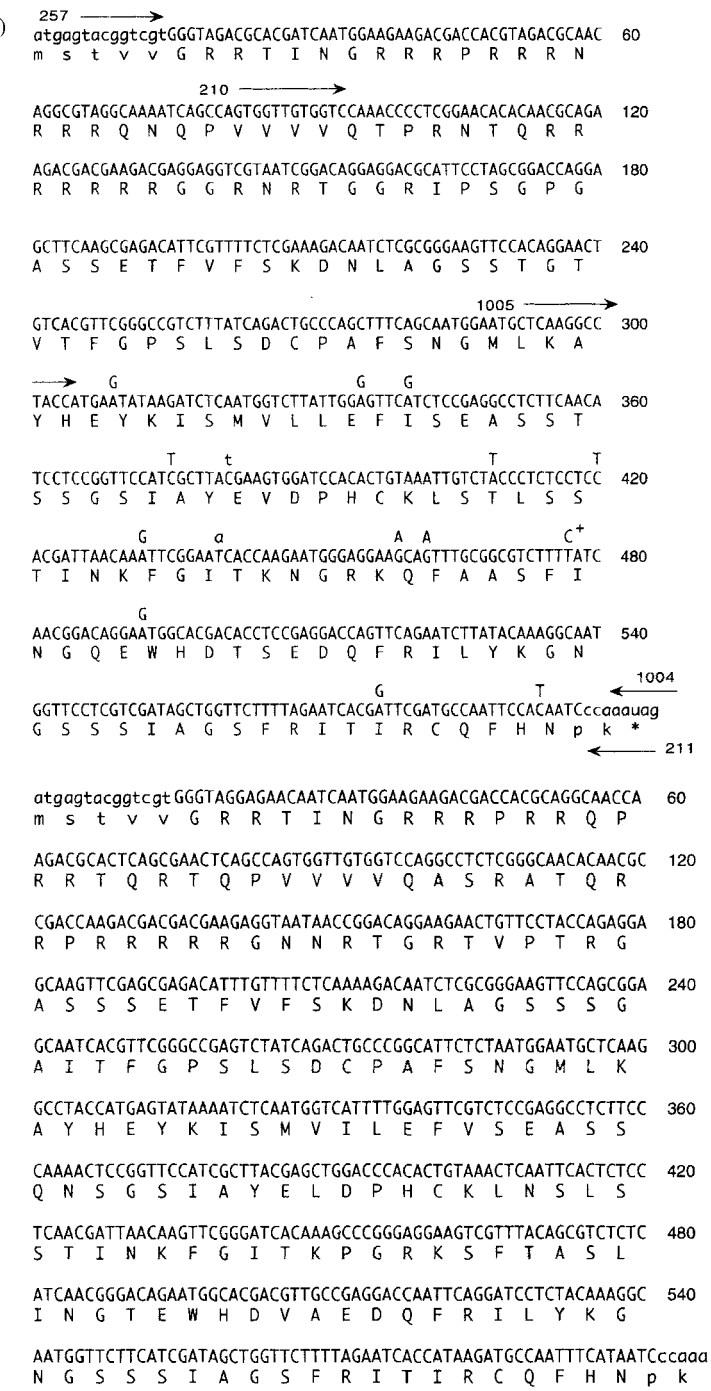

Fig. 1. Nucleotide sequence of the putative coat protein genes of luteovirus isolates IC (*a*) and L (*b*). Deduced amino acid sequences of the putative coat proteins are indicated beneath each nucleotide sequence. The positions of the primers are shown by horizontal arrows. Nucleotide and amino acid sequence attributable to the sequence of the primers are shown in lower case. In Fig. 1*a* nucleotides are shown above the sequnce where they differed from the sequence of IC in individual clones of cDNA from virus purified from chickpea plants harvested in 1993–94. Nucleotides in upper case are those for variant IC4, those in lower case were in the sequences of the other variants; + indicates that C was present in this position in the sequences of IC4 and the other variants.

material). It is interesting to note that distinct patterns of reaction were observed in successive seasons only at one location (IAC).

RT/PCR analysis

Group-specific oligonucleotide primers (#210 and #211) have been used previously to amplify part of the coat protein gene of a range of luteoviruses (Robertson *et al.*, 1991). Therefore, these two primers were used in the initial experiments for RT/PCR analysis of RNA extracted from plants infected with isolates IC or L, and of RNA extracted from the purified virus preparation that was used in aphid transmission studies (i.e. collected from IAC and Junagadh during 1992–93, see Table 1). A DNA product of approximately 550 bp was produced by RT/PCR of RNA from these samples. In subsequent RT/PCR experiments, primer #211 and an upstream primer (#257) identical to a sequence starting at the initiation codon of the PLRV coat protein were used to amplify the entire coat protein gene. The approximately 600 bp PCR fragment was cloned into pGEM-T. Individual clones derived from two independent RT/PCR reactions for each sample were sequenced in both orientations. The nucleotide sequences of the coat protein genes of isolates IC and L are shown in Fig. 1, together with the deduced amino acid sequences of their putative coat proteins. The EMBL Accession numbers are 11530 and 11531 respectively. Two types of sequences were obtained from the purified virus preparation of 1992–93 material from IAC that was used in aphid transmission studies. These sequences matched with those of either IC or L. However, samples from Junagadh yielded only one sequence which was 99% identitical to the coat protein gene sequence of isolate IC (data not shown).

The upstream 'universal' primer (#210) was a poor match to the coat protein gene of BLRV and SDV, strains of which have been reported to be associated with chickpea stunt disease (Bosque-Perez & Buddenhagen, 1990; Carazo *et al.*, 1993). This primer would be ineffective in a PCR reaction to amplify coat protein gene sequences of either virus, if present, in the chickpea samples. Therefore, new primers were designed taking into account the nucleotide sequences of the coat protein genes of all luteoviruses known at present (Mayo & Ziegler-Graff, 1996). This pair of primers (#1004 and #1005) was used in RT/PCR to amplify cDNA from RNA of purified virus obtained from chickpea plants showing stunt symptoms collected at IAC during 1993–94. Six clones were obtained by cloning the PCR products of approximately 320 bp in pGEM-T. The sequences of the cDNA inserts in five clones, excluding the sequences of the primers, were largely identical to the 3′-most 293 nucleotides of the coat protein gene of isolate IC. The nucleotide sequences of these clones differed from that of IC at three positions (nucleotides 381, 440 and 477) of which that at position 440 altered the encoded amino acid sequence (Fig. 1*a*). However, the sequence of a sixth clone (variant IC4) was different from that of isolate IC at 13 positions (nucleotides 309, 335, 340, 375, 409, 420, 432, 459, 462, 477, 492, 577 and 594) (Fig. 1*a*). Four changes (at positions 335, 340, 409 and 577), altered the amino acid sequence encoded. Isolate IC4 is 96% identical isolate IC and the other variants in both nucleotide and amino acids sequence. The results show that minor coat protein variants of isolate IC are present in chickpea plants showing stunt disease symptoms.

Thus the results of nucleotide sequence analysis support the serological data in that isolates IC and L are distinct and that samples of diseased chickpeas collected at IAC in 1992–93 contained a mixture of these two isolates. Isolate IC seems to be widespread and to appear in successive years whereas isolate L appeared only in one year and only at IAC. The RT/PCR analysis of diseased chickpeas collected at IAC in 1993–94 showed that sequence variants of isolate IC were present. Isolates IC and L differ both in their reactions with certain MAbs and

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BLRV   MVAR-GKRVV-----VRQLQTRARRRLPVVLATAPVRPQRKRRQRGRNNKPRGGNGFARRSSQVHEF
IC     mstvvGRRTINGRRRPRRRNRRRQN-QPVVVVQTPRNTQRRRRRRRGGRNRTGGRIPSGPGASSETF
BWYV   MNTVVGRRIINGRRRPRRQTRRAQRPQPVVVVQTSRATQRRPRRRRRGNNRTGRTVPTRGAGSSETF
L      mstvvGRRTINGRRRPRRQPRRTQRTQPVVVVQASRATQRRPRRRRRGNNRTGRTVPTRGASSSETF

BLRV   VFSKDNLNGNSKGSITFGPSLSECKPLADGILKAYHEYNITNVELAYITEASSTSSGSIAYELDPHL
IC     VFSKDNLAGSSTGTVTFGPSLSDCPAFSNGMLKAYHEYKISMVLLEFISEASSTSSGSIAYEVDPHC
BWYV   VFSKDNLAGSSSGAITFGPSLSDCPAFSNGMLKAYHEYKISMVILEFVSEASSQMSGSIAYELDPHC
L      VFSKDNLAGSSSGAITFGPSLSDCPAFSNGMLKAYHEYKISMVILEFVSEASSQNSGSIAYELDPHC

BLRV   KNTTIQSKINKFSITKSEKKKFSRKAINGQAWHDTSEDQFRILYEGNGDAKIAGSFRVTIKVLTQNPK
IC     KLSTLSSTINKFGITKNGRKQFAASFINGQEWHDTSEDQFRILYKGNGSSSIAGSFRITIRCQFHNpk
BWYV   KLNSLSSTINKFGITKPGKRAFTASYINGTEWHDVAEDQFRILYKGNGSSSIAGSFRITIKCQFHNPK
L      KLNSLSSTINKFGITKPGRKSFTASLINGTEWHDVAEDQFRILYKGNGSSSIAGSFRITIRCQFHNpk
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Fig. 2. Amino acid sequence similarities between the coat proteins of BLRV and isolate IC, between isolate IC and BWYV, and between BWYV and isolate L. The alignment was generated by using CLUSTALV. Vertical bars indicate the positions of residues that are identical in adjacent sequences. Amino acids shown in lower case are encoded by the primers used.

in the sequences of their coat proteins. The coat proteins were 82% identical which suggests that the isolates represent distinct viruses (see below).

Comparisons of chickpea luteovirus coat proteins with those of other luteoviruses

Fig. 2 shows an alignment of the coat protein amino acid sequences of IC and L isolates with those of BLRV and BWYV, which have been identified previously as causal agents of chickpea stunt disease. Table 2 shows the percentage identities of the coat proteins of isolates IC and L with those of a range of luteoviruses. Both IC and L sequences differ a lot (55% or 53% identical) from that of BLRV. However, the IC and L sequences each resemble that of BWYV more than those of any other luteovirus. As the L sequence was 94% identical to that of BWYV, L may best be regarded as a strain of BWYV whereas isolate IC is sufficiently distinct (< 82% identical) from all the other luteoviruses for it to be considered as a distinct virus.

de Miranda *et al.* (1995) classified the coat proteins of a number of isolates of BWYV and BMYV into several groups. The coat protein sequence of isolate L was between 88% and 93% identical to the sequences analysed by de Miranda *et al.* (1995) but was not noticeably more similar to any one than to any other. The coat protein sequence of isolate IC was between 78% and 81% identical to the same range of sequences.

Table 2. *Percent identities in amino acid sequence between the coat proteins of isolates IC and L and those of other luteoviruses*

	IC	L
IC	100	82
L	82	100
BWYV	80	94
BMYV	77	88
GRAV	68	66
CABYV	66	66
PLRV	61	60
RPV	61	64
SDV	61	58
SPLSV	60	59
BLRV	55	53
MAV	45	44

Discussion

The primary aim of the work described was to assess the diversity of luteoviruses associated with chickpea stunt disease. From the results of assays of plant extracts with a panel of MAbs and the nucleotide sequence of putative coat protein gene sequences isolated from infected plants, we were able to infer the presence of two distinct luteoviruses. Previous diagnoses of luteoviruses infecting chickpea were based on serological reactions (Kaiser & Danesh, 1991; Reddy *et al.*, 1979; Bosque-Perez & Buddenhagen, 1990; Carazo *et al.*, 1993; Horn *et al.*, 1996). The results of the present study illustrate that for purposes of identification of viruses belonging to the genus *Luteovirus*, it is necessary to combine the use of serological methods, even when using MAbs, with more precise characterisation such as nucleotide sequencing. The results also show that sequence analysis can resolve viruses in a mixed infection, although it is not clear how sensitive the method would be to a disproportion between the components in such a mixed infection.

Variability in serological reactions and in coat protein gene sequences of luteoviruses associated with chickpea stunt disease was observed in only one location, namely IAC. Since chickpea is grown as an annual crop, it is possible that the disease invades crops from a reservoir in other host species each year. Thus the abundance of different luteoviruses in, and the proximity of chickpea crop to, these reservoirs presumably determines which virus, or what proportion of the different viruses, occurs in each year. *M. persicae* did not transmit isolates IC and L with equal efficiency and the prevalence of different vector species may also contribute to the relative abundance of the luteoviruses associated with the disease. Surveys conducted in different regions of India for two consecutive years have shown that isolates similar to IC are widely distributed and predominant in chickpeas (Table 1).

The present study raises several questions. For example, it remains to be determined if individual luteoviruses can induce the symptoms of stunt disease or if the symptoms are induced as a result of synergistic interaction between different luteoviruses. Experiments to answer this question will be possible only when the aphid vectors for IC isolates are identified. Nevertheless, the availability of means for the detection and differentiation of the two viruses as shown in this paper will facilitate studies of their ecology and epidemiology, as well as in the detection and exploitation of virus-resistant germplasm in breeding programmes to improve the chickpea crop.

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