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Viral diseases affecting chickpea crops in Eritrea

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Summary. A survey to identify virus diseases affecting chickpea crops in the major production areas of Eritrea was conducted during November 2005. The survey covered 31 randomly selected chickpea fields. Virus disease incidence was determined on the basis of laboratory testing of 100–200 randomly collected samples from each field against antisera of 9 legume viruses. Serological tests indicated that the Luteoviruses were the most common, with an overall incidence of 5.6%, followed by *Faba bean necrotic yellows virus* (FBNYV, genus *Nanovirus*, family *Nanoviridae*) (4.1%) and Chickpea chlorotic dwarf virus (CpCDV, genus *Mastrevirus*, family *Geminiviridae*) (0.9%). The reverse transcription polymerase chain reaction (RT-PCR) test showed that the most common luteoviruses in Eritrea are *Chickpea chlorotic stunt virus* (CpCSV) followed by *Beet western yellows virus* (BWYV, genus *Polerovirus*, family *Luteoviridae*). Based on the field symptoms observed, 29 fields had, at the time of the survey, a virus disease incidence of 1% or less and only two fields had an incidence of about 5%, whereas on the basis of laboratory testing, 19 fields had more than 6% virus incidence (three of these had an incidence of 29.5, 34.5 and 40.5%). This is the first survey of chickpea viruses in Eritrea and the first report of BWYV, CpCDV, CpCSV and FBNYV naturally infecting chickpea in Eritrea.

Key words: *Beet western yellows virus,* Chickpea chlorotic dwarf virus, *Chickpea chlorotic stunt virus, Faba bean necrotic yellows virus.*

Introduction

Chickpea (*Cicer arietinum* L.) is an ancient crop that has been grown in India, the Middle East and parts of Africa for many years. It is the most important pulse crop in Eritrea, with an estimated cultivated area of 18,590 ha during the 2005–2006 growing season (FAOSTAT, 2008), mostly of the 'desi' type. Chickpea is an important source of protein in the human diet and plays a significant role in farming systems. It ranks as the third most important non-oilseed grain legume in the world, after Phaseolus beans and peas.

Chickpea is host to a wide range of fungal and viral diseases, which can cause yield losses in disease-favourable environments. Six viruses are known to affect chickpea in different parts of the world (Makkouk *et al*., 2003; Kumari *et al*., 2008): *Alfalfa mosaic virus* (AMV, genus *Alfamovirus*, family *Bromoviridae*), *Bean leafroll virus* (BLRV, genus *Luteovirus*, family *Luteoviridae*), *Beet western yellows virus* (BWYV, genus *Polerovirus*, family *Luteoviridae*), Chickpea chlorotic dwarf virus (CpCDV, genus *Mastrevirus*, family *Geminiviridae*), *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family *Bromoviridae*) and *Faba bean necrotic yellows virus* (FBNYV, genus *Nanovirus*, family *Nanoviridae*). Recently, *Chickpea chlorotic stunt virus* (CpCSV, genus *Polerovirus*, family *Luteoviridae*) has also been reported to affect chickpea in Ethiopia (Abraham *et al*., 2006).

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Improved resistance to disease could increase the yield stability of newly bred varieties. However, in order to set priorities for the development of new varieties and for disease management, quantitative data are needed on the occurrence of viruses in the different cultivation areas.

In Eritrea, viral diseases of chickpea have not been extensively studied and no information is available on their incidence. The main objective of this study was to determine which viruses affect chickpea in the major production areas of Eritrea and to determine their incidence and relative importance using diagnostic tests with virus-specific antibodies.

Materials and methods

Field visits and sample collections

Field visits and collection of samples were conducted during November 13–22, 2005, when the plants were at the flowering/pod setting stage. Thirty-one fields belonging to chickpea farmers were randomly selected. The exact location of the fields is shown in Fig. 1. Each field was evaluated using a standard format, recording field location, crop condition, growth stage, virus disease symptoms, and aphid populations.

Virus disease incidence in each field was determined on the basis of visual symptoms and by counting the percentage of infected plants at different, randomly selected locations in the field. From each field, two types of samples were collected; 1–25 samples from symptomatic plants, and 100–200 samples from randomly collected plants with or without symptoms. Samples were placed in labelled plastic bags and brought to the laboratory for testing. Testing for key viruses was done at the Plant Pathology Laboratory at Halhala Station, NARI, Eritrea and at the Virology Laboratory of ICARDA, Aleppo, Syria.

A total of 522 chickpea samples with symptoms suggestive of virus infection (chlorosis, stunting, necrosis, reddening), and 5860 randomly collected samples from 31 chickpea fields were tested.

Laboratory tests

Tissue-blot immunoassay (TBIA) and antisera used

All samples were tested for viruses using the tissue-blot immunoassay (TBIA) technique (Makkouk and Comeau, 1994; Makkouk and Kumari, 1996) against a battery of polyclonal and monoclonal antibodies. The ICARDA Virology Laboratory provided rabbit polyclonal antisera for the following viruses: CpCDV (Kumari *et al*., 2006b), CMV, AMV, *Bean yellow mosaic virus* (BYMV) and *Pea seed-borne mosaic virus* (PSbMV) (genus *Potyvirus*, family *Potyviridae*). A monoclonal antibody to detect FBNYV (3-2E9) was provided by A. Franz (Franz *et al*., 1996), and a broad-spectrum legume-luteovirus monoclonal antibody (5G4) was provided by L. Katul (Katul, 1992), BBA, Braunschweig, Germany.

To identify the luteoviruses affecting chickpea in Eritrea, infected samples that gave a positive reaction to the broad-spectrum monoclonal antibody 5G4 were further tested against three specific monoclonal antibodies: one for BWYV (Agdia Inc., IN, USA), one for BLRV (4B10; Katul, 1992), and one for *Soybean dwarf virus* (SbDV, genus *Luteovirus*, family *Luteoviridae*) (ATCC PVAS-650).

PCR analysis

RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) analysis. Thirteen chickpea samples (from 12 fields) that reacted serologically with Luteovirus McAb (5G4) (3 of them reacted with BWYV McAb) were selected for further testing by RT-PCR. Total RNA was extracted from all samples using components of the Plant RNeasy Kit from (QIAGEN GmbH, Hilden, Germany, Cat No. 74904) following the method of MacKenzie *et al*. (1997) and Nassuth *et al*. (2000). RNAs were detected (MacKenzie, 1997; Nassuth *et al*., 2000) using a one-step RT-PCR kit from Invitrogen Australia (Melbourne, Australia, Cat No. 12574-026). The amplified fragments were separated in 1% agarose gel containing ethidium bromide by electrophoresis in TBE buffer using 15 μl of the PCR mixture, and visualized with a UV transilluminator. Table 1 summarizes the luteovirus primers used in the study (prepared by GeneWorks Pty Ltd). Lu_1+Eco and Lu_4+Eco primers were used instead of Lu_1 and Lu_4 (Robertson *et al*., 1991). The main objective of adding the Eco sequence to the primers was to increase the annealing temperature.

DNA extraction and polymerase chain reaction (PCR) analysis. Five chickpea samples (from 5 fields) that reacted serologically with the FBNYV monoclonal antibody were selected for further testing by PCR. Total DNA was extracted

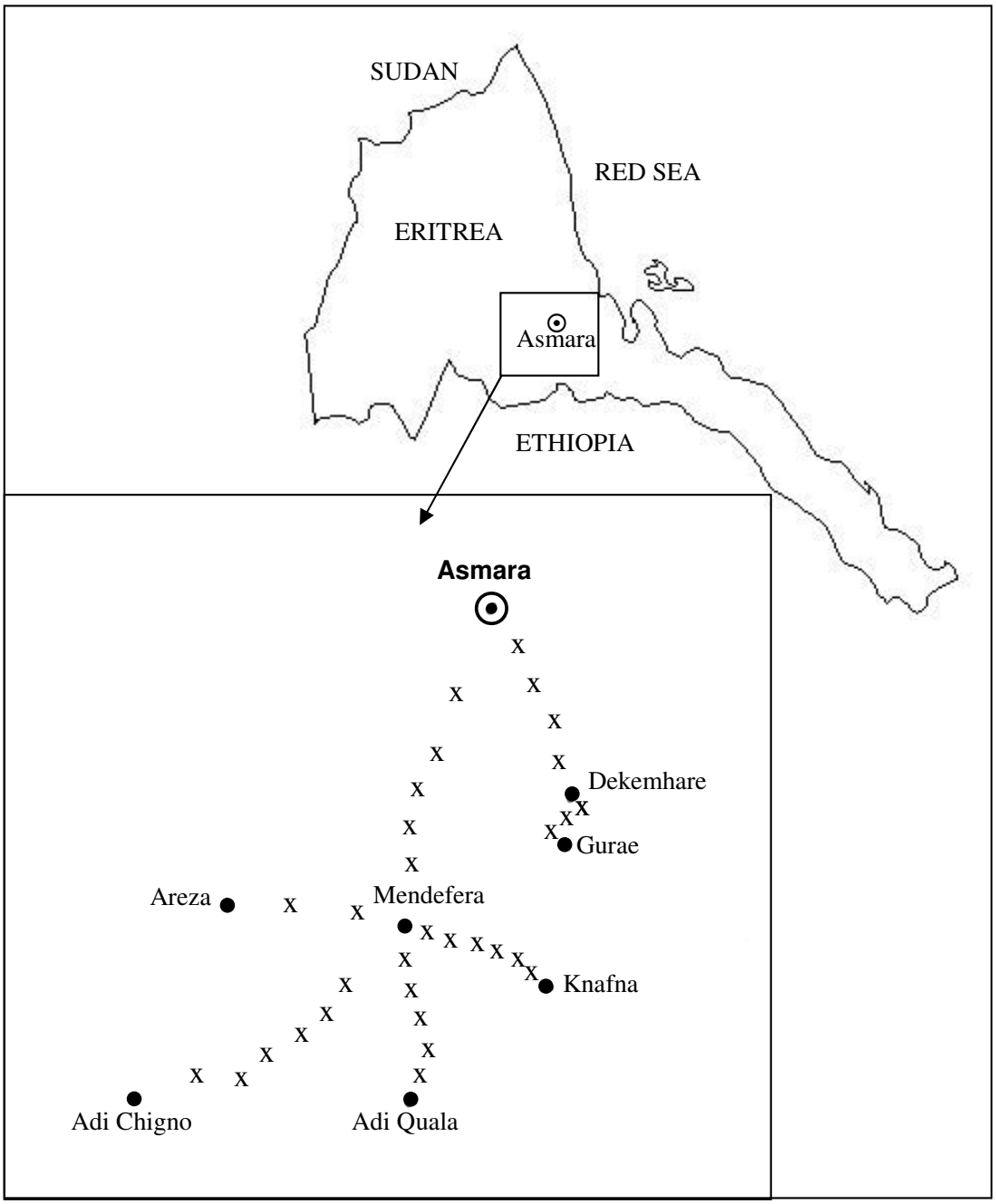

Fig. 1. Map of Eritrea showing the location of chickpea fields (x) surveyed in November 13–22, 2005.

from all samples using the reagents provided and the methods described in the DNeasy Plant Mini Kit (Qiagen, Cat No. 69104). Platinum® *Taq* DNA Polymerase (Invitrogen Australia, Cat No. 10966) was used for PCR according to manufacturer's instructions except that the total reaction volume was 25 μl, and 1.0 unit of enzyme was used per reaction. The amplified DNA fragments were analyzed electrophoretically by running 15 μl of the PCR mixture on a 1% agarose gel in TBE buffer containing ethidium bromide and visualized with a UV transilluminator. The nanovirus primer sets used in the study are listed in Table 1.

Results

Field observations

The virus-like disease symptoms most commonly observed in chickpea fields were chlorosis, stunting, and reddening of the leaves. Based on the symptoms

Table 1. Primers used for the detection of *Luteoviruses* and *Nanoviruses* by PCR.

Primers[a]	Primer Sequence[b]	Primer size (bp)	Product size (bp)	Reference
Primers used for RT-PCR	**amplification of *Luteoviruses***			
Lu_1 + Eco side	5′-GAATTCCCAGTGGTTRTGGTC-3′	21	530	Robertson *et al*., 1991
Lu_4 + Eco side	5′-GAATTCGTCTACCTATTTGG-3′	20		
BWYV CP-F	5′-ATGAATACGGTCGTGGGTAC-3′	20	429	Kumari *et al.,* 2006a
BWYV CP-R	5′-GATAGTTGAGGAAAGGGAGTTG-3′	22		
SbDV rep-F	5′-AGGCCAAGGCGGCTAAGAG-3′	19	440	Kumari *et al.,* 2006a
SbDV rep-R	5′-AAGTTGCCTGGCTGCAGGAG-3′	20		
BLRV-3 (Ortiz)	5′-TCCAGCAATCTTGGCATCTC-3′	20	391	Ortiz *et al*., 2005
BLRV-5 (Ortiz)	5′-GAAGATCAAGCCAGGTTCA-3′	19		
CpCSV-F	5`-TAGGCGTACTGTTCAGCGGG-3`	20	413	This study. Based on Abraham *et al*., 2006
CpCSV-R	5`-TCCTTTGTCCATTCGAGGTGA-3`	21		
Primers used for PCR of	**amplification *Nanoviruses***			
Nano F103	5′-ATTGTATTTGCTAATTTTA-3′	19	771–775	Kumari *et al.,* 2008
Nano R101	5′-TTCCCTTCTCCACCTTGT-3′	18		
SCSV F	5′-TWC YGG GTA ACA CGG TTT GA-3′	20	700	Kumari *et al*., 2008
SCSV R	5′-CGG AGA CAT ATG ACG TCA-3′	18		
FBNYV C5-F	5′-TACAGCTGTCTTTGCTTCCT-3′	20	666	Kumari *et al*., 2008
FBNYV C5-R	5′-CGCGGAGTAATTAAATCAAAT-3′	23		
MDV CP-F	5′-TCTCTCTATAAAAGCTGTTA-3′	20	608	Kumari *et al*., 2008
MDV CP-R	5′-AAATGATTGTTGATTTCATT-3′	20		

[a] BWYV, *Beet western yellows virus*; SbDV, *Soybean dwarf virus*; BLRV, *Bean leafroll virus*; CpCSV, *Chickpea chlorotic stunt virus*; SCSV, *Subterranean clover stunt virus*; FBNYV, *Faba bean necrotic yellows virus;* MDV, *Milk vetch dwarf virus*; rep, replicate gene; CP, coat protein gene.
[b] R=AG, W=AT, Y=CT.

observed, virus disease incidence was less than 1% in 29 of the 31 fields surveyed, and only about 5% in the other two fields. No aphids colonizing plants were observed in any of the fields.

Virus identification and incidence based on laboratory testing

Serological results

Laboratory testing of the 5860 randomly collected chickpea samples indicated that the luteoviruses were the most frequent, with an overall incidence of 5.6%, followed by FBNYV (4.1%) and CpCDV (0.9%) (Table 2). Of the 522 symptomatic samples tested, only 211 samples reacted with 5G4 (luteoviruses), 49 samples with CpCDV and 43 samples with FBNYV (Table 2).

When the 211 samples that had shown a positive reaction with the broad-spectrum legume-luteovirus monoclonal antibody 5G4 were re-tested against the three specific monoclonal antibodies (BWYV, BLRV and SbDV), only 12 of them reacted with BWYV-specific monoclonal antibody. All tested samples were negative to BLRV, SbDV, BYMV, PSbMV, CMV and AMV.

PCR results

When 5 samples that reacted serologically with FBNYV McAb were further tested by PCR using 4 Nanovirus primer pairs, all samples gave amplicons with the generic nanovirus (M-Rep component) (F103 and R101) and FBNYV (C5F and C5R) primers. By contrast, none of these samples produced

Table 2. Results of laboratory tests on chickpea samples randomly collected or with symptoms suggestive of virus infection, from 31 fields in Eritrea during November 13–22, 2005. Viral identification was based on serological reactions (TBIA). Figures in brackets represent virus incidence (%).

Region	Sample collection method	No. of fields surveyed	No. of samples tested	No. of samples found positive to[a]			Average incidence (%)[b]
				FBNYV	5G4	CpCDV	
Asmara-Mendefera	Symptoms	5	77	4	38	7	7.35
	Random		830	9	50	2	
Mendefera - Adi Quala	Symptoms	5	81	8	29	12	8.62
	Random		870	21	47	7	
Mendefera – Adi Chigno	Symptoms	6	103	26	33	13	11.98
	Random		1160	96	42	9	
Asmara – Dekemhare	Symptoms	4	57	1	21	6	8.05
	Random		770	12	36	14	
Dekemhare – Gurae	Symptoms	3	59	2	24	2	7.3
	Random		600	11	29	4	
Mendefera – Areza	Symptoms	2	34	2	14	4	7.80
	Random		410	13	12	7	
Mendefera – Knafna	Symptoms	6	111	0	52	5	13.52
	Random		1220	76	110	11	
Total	Symptoms	31	522	43 (8.2)	211 (40.4)	49 (9.4)	10.55
	Random		5860	238 (4.1)	326 (5.6)	54 (0.9)	

[a] All samples were negative to AMV, BLRV, BYMV, CMV, PSbMV and SbDV. Virus acronyms used are: AMV, *Alfalfa mosaic virus*; BLRV, *Bean leaf roll virus*; BYMV, *Bean yellow mosaic virus*; BWYV, *Beet western yellows virus*; CpCDV, *Chickpea chlorotic dwarf virus*; FBNYV, *Faba bean necrotic yellows virus*; CMV, *Cucumber mosaic virus*; PSbMV, *Pea seed-borne mosaic virus*; SbDV, *Soybean dwarf virus*; 5G4, a broad spectrum monoclonal reacting with all legume luteoviruses.

[b] Total incidence was calculated only from samples collected at random.

any amplification products when MDV- and SCSV-specific primers were used.

When 13 Luteovirus samples were tested by RT-PCR using four Luteovirus primer pairs, all produced amplicons when the generic Luteovirus (Lu1+Eco; Lu4+Eco) primers were used. In addition, only three samples (which reacted with BWYV MAb) yielded PCR products with the BWYV primers. Ten samples that reacted serologically with the broad-spectrum legume-luteovirus monoclonal antibody 5G4 produced amplicons when CpCSV primers were used (Fig. 2). In no case did BLRV-, SbDV- or BWYV-specific primers produce amplification products from these 10 Eritrean samples that gave amplicons with CpCSV primers.

Comparison of field-observed incidence with laboratory test results

Figure 3 summarizes virus incidence in chickpea fields surveyed based on (i) virus-like symptoms observed in the field, and (ii) results of laboratory testing of randomly collected samples. Virus disease incidence assessment based on visual symptoms underestimated virus incidence

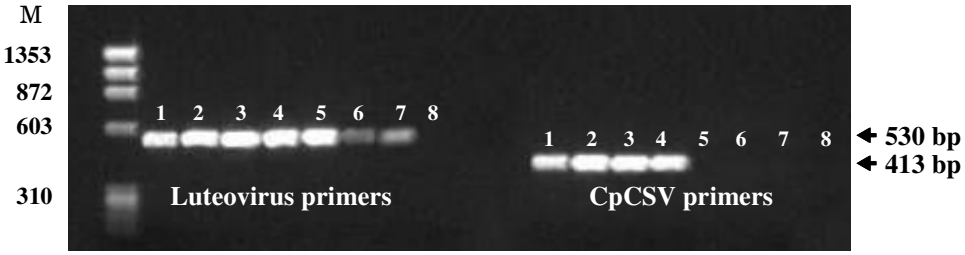

Fig. 2. Detection of *Chickpea chlorotic stunt virus* (CpCSV) from Eritrea by RT-PCR using Luteovirus (Lu_1+Eco and Lu_4+Eco) (left) and CpCSV (right) primers. 1-4, CpCSV isolate from Eritrea; 5, *Beet western yellows virus*; 6, *Bean leafroll virus*; 7, *Soybean dwarf virus*; 8, negative control; M, DNA molecular weight markers ladder (IX, from Roche, Cat no. 1449 460).

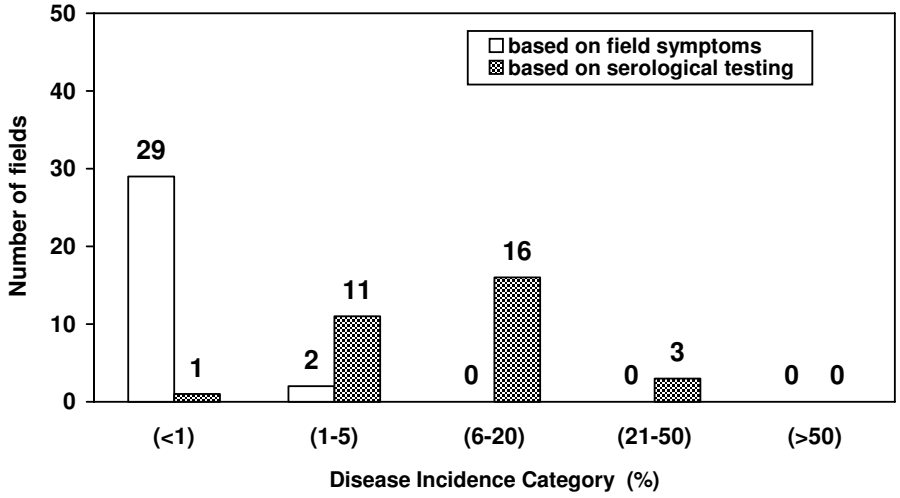

Fig. 3. Comparison between virus incidence in chickpea crop based on symptoms observed in the field and as determined by laboratory testing of randomly collected samples during a survey conducted in Eritrea, November 13–22, 2005.

as determined by laboratory testing of randomly collected samples. For examples, no chickpea fields were in the higher than 6% incidence category based on visual observation, whereas lab testing of randomly collected samples revealed that 19 fields were in this category.

Discussion

Laboratory testing of randomly collected chickpea samples showed that about 39% of the fields surveyed had a virus disease incidence level of 6% or less. The yields of such fields will most likely be only slightly affected by viral infections. In 61% of the fields, virus disease incidence was in the 6–50% range (in three fields it was 29.5, 34.5 and 40.5%). Such fields will most likely suffer yield loss to various degrees.

Both field observations and the laboratory tests indicated that the major virus problems on chickpea in Eritrea were caused by the luteoviruses (e.g. CpCSV, BWYV) and FBNYV. It was expected that percentage yield losses would be very close to the percentage incidence of the persistently transmitted viruses such as BWYV, FBNYV and CpCDV, mainly because legume plants infected with these viruses produce few or no pods (Bos *et al*., 1988; Franz *et al*., 1997). The incidence of these viruses in the fields averaged 10.5%, suggesting a yield loss in chickpea production in Eritrea of about 10.5%. This information suggests that these viruses deserve attention when developing strategies to improve chickpea production in Eritrea. In addition, chickpea should be screened for BWYV, CpCSV and FBNYV resistance. Local as well as exotic germplasm can be evaluated for such purpose.

Even though no aphids were observed in any of the fields, the fact that some fields had a very high incidence of persistently aphid-transmitted viruses (mostly luteoviruses) suggested that winged aphids had been active when the crops were at the early stages of growth.

FBNYV has been reported to infect food legumes in many countries in WANA (Makkouk *et al*., 1992; Katul *et al*., 1993; Horn *et al*., 1995; Tadesse *et al*., 1999; Najar *et al*., 2000). Even though this virus reaches epidemic levels in some countries, such as Egypt (Makkouk *et al*., 1994), its incidence in Eritrea, as revealed by this study, was low (4.1%). Moreover, the survey was conducted during November, when 70% of the fields were approaching maturity and around 30% were still at the flowering stage. The FBNYV incidence levels here reported can be expected to increase further towards the end of the growing season if environmental conditions are favourable for the multiplication and spread of the aphid vector population. However, because of the damage this virus causes, it needs to be monitored more closely in the coming years.

CpCSV was recently reported to occur naturally on chickpea in Ethiopia (Abraham *et al*., 2006). In the present study, this virus was detected for the first time in chickpea in Eritrea; consequently, further work would be useful to determine the incidence and relative importance of this virus, and to identify the aphid species that transmit it naturally.

A total of 199 out of the 211 chickpea samples tested reacted with the legume broad-spectrum monoclonal antibody 5G4 but not with any of the specific antibodies used in this study. However, 9 of these samples were infected with CpCSV when tested by RT-PCR. This indicates either that all these samples are infected with CpCSV, or that luteoviruses other than CpCSV, BWYV, BLRV and SbDV, which can infect chickpea, occur in Eritrea.

The results indicated that laboratory testing is essential for an accurate assessment of virus incidence in the field. Of the chickpea fields surveyed, 61% of those that were placed in a higher than 6% virus disease incidence category by laboratory testing, were placed in a lower than 6% incidence category by visual field inspection. An assessment based on field inspection alone is therefore likely to under-estimate the economic crop loss caused by viral diseases.

This survey indicated for the first time that BWYV, CpCSV, FBNYV and CpCDV naturally infect chickpea crop in Eritrea, and this is the first report of CpCSV outside Ethiopia.

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