

## Survey for chickpea and lentil virus diseases in Ethiopia

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**Summary.** Chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medik.) are among the major cool-season food legumes grown in Ethiopia. Fungal and viral diseases are important factors limiting production of these two crops in the country. As there is no systematic study on the virus diseases of these crops, a survey to identify virus diseases of chickpea and lentil crops in Shewa province, Ethiopia, was conducted during November 14-23, 1998. The survey covered a random selection of 33 chickpea and 32 lentil fields. Virus disease incidence was determined by laboratory testing 100-200 randomly collected samples from each field with antisera of 12 viruses. In chickpea fields, beet western yellows polerovirus (BWYV) was most frequently found and in lentil fields, pea seed-borne mosaic potyvirus (PSbMV) was the most common, followed by luteoviruses [BWYV and soybean dwarf virus (SbDV)]. Laboratory testing of random samples, showed that 5 chickpea and 11 lentil fields had a virus disease incidence of 21% or higher. The highest virus disease incidence in a single field was 58.5% for lentil (PSbMV) and 41.3% for chickpea (BWYV). Other viruses detected more rarely, were faba bean necrotic yellows nanovirus (FBNYV) and broad bean wilt fabavirus (BBWV) in chickpea; FBNYV, broad bean stain comovirus (BBSV), bean yellow mosaic potyvirus (BYMV) and cucumber mosaic cucumovirus (CMV) in lentil.

**Key words:** viruses, *Cicer arietinum* L., *Lens culinaris* Medik., Ethiopia.

### Introduction

Chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medik.) are important cool-season food legumes grown in Ethiopia. Some 126,500 tonnes of chickpea seed are produced annually on nearly 147,900 ha, with an average yield of 860 kg/ha. The total area under lentil cultivation during the 1996-7 cropping season was 52,810 ha, with an average seed yield of 643 kg/ha. Shewa is the largest producer, accounting for about 50% of the country's total area planted with these crops; other provinces (regions) producing significant

quantities are Gondar, Gojam, Tigray, and Wello (Central Statistical Authority, 1997).

Various fungal and viral diseases limit the production of these two crops. Chickpea stunt disease caused by bean leaf roll virus (BLRV) has been recorded on chickpea in different countries of the world (Nene *et al.*, 1996). Lentil yellows has been reported from West Asia and North Africa, including Ethiopia, Syria and Algeria (Beniwal *et al.*, 1993). However these viruses were mostly identified on the basis of field symptoms only.

Beet western yellows virus (BWYV) is reported

on chickpea from different countries, including Asia and Africa (Horn *et al.*, 1995; Nene *et al.*, 1996; Fortass *et al.*, 1997), and is known to have a wide host range that includes more than 100 species in at least 21 dicotyledonous plant families (Duffus, 1972).

Viral diseases of chickpea and lentil in Ethiopia have not been extensively studied and identified. The purpose of this survey was to identify these diseases, with emphasis being placed on Shewa province, the most important chickpea and lentil growing province in Ethiopia.

## Materials and methods

### Field visits and sample collection

The survey was conducted during November 14-23, 1998 when chickpea and lentil crops were at the flowering-podding growth stage. Field visits to observe virus disease symptoms were made to 33 chickpea and 32 lentil fields in ten districts of east, west and north Shewa (Fig. 1). Shewa lies approximately between 60 and 110 latitude north and 360 and 410 longitude east.

Fields selected were at predetermined distances from each other, and each field was evaluated using a standard format. Viral disease incidence in each field was determined by inspection of symptoms and by calculating the percentage of infected plants at different locations in the field. From each field, two types of samples were collected; from 20-25 plants with symptoms suggestive of viral infection, and from 100-200 randomly selected plants.

### Laboratory tests

A total of 7770 random samples (3100 chickpea and 4670 lentil) and 1493 symptomatic samples (736 chickpea and 757 lentil) was assayed for viruses using the tissue blot immunoassay (TBIA) (Makkouk and Comeau, 1994) at the Pathology Laboratory of Debre Zeit Agricultural Research Center.

All samples were tested against a battery of polyclonal and monoclonal antibodies. Rabbit polyclonal antiserum for chickpea chlorotic dwarf geminivirus (CCDV) was provided by D.V.R. Reddy, ICRISAT, India. Broad bean stain comovirus (BBSV), pea seed-borne mosaic potyvirus (PSbMV), bean yellow mosaic potyvirus

(BYMV), broad bean wilt fabavirus (BBWV), broad bean mottle bromovirus (BBMV), cucumber mosaic cucumovirus (CMV), pea enation mosaic virus (PEMV), alfalfa mosaic alfamovirus (AMV), and broad bean true mosaic comovirus (BBTMV) polyclonal antisera were supplied by the Virology Laboratory of ICARDA, Aleppo, Syria. In addition, two monoclonal antibodies for the detection of faba bean necrotic yellows nanovirus (FBNYV) and a broad-spectrum legume luteovirus (5G4) were provided by A. Franz and L. Katul, BBA, Braunschweig, Germany.

To identify the individual luteoviruses affecting chickpea and lentil crops in the country, the infected samples that gave a positive reaction to the broad-spectrum monoclonal 5G4 were further screened against four specific monoclonals, BWYV (ATCC PVAS-647), soybean dwarf virus (SbDV) (ATCC PVAS-650), BLRV (6G4), and 3B11, a monoclonal that reacts with either BLRV or SbDV (Katul, 1992), and against the polyclonal chickpea luteovirus (CPLV) supplied by D.V.R. Reddy, ICRISAT.

## Results

### Virus disease incidence observed in the fields

The virus disease symptoms most commonly observed in the chickpea and lentil fields were chlorosis, stunting, yellowing and reddening of the leaves. Virus disease incidence based on symptoms observed is summarized in Fig. 2. Fifty percent of the chickpea fields inspected (16 fields) had a virus incidence of 6% or higher, whereas only one lentil field had such a high incidence.

### Virus identification and incidence on the basis of laboratory testing

Laboratory testing of randomly collected lentil samples revealed that PSbMV was the most common virus, with an overall average of 9.9%, followed by the luteoviruses and FBNYV (Table I). The incidence of viruses CMV, BBSV and BYMV was extremely low (around 0.02%). Laboratory testing of randomly collected chickpea samples indicated that the luteoviruses were the most common, with an overall incidence of 10.6%, whereas FBNYV and BBWV were rare (Table II). The highest virus disease incidence recorded in a lentil field was 58.5% with PSbMV at Deneba and

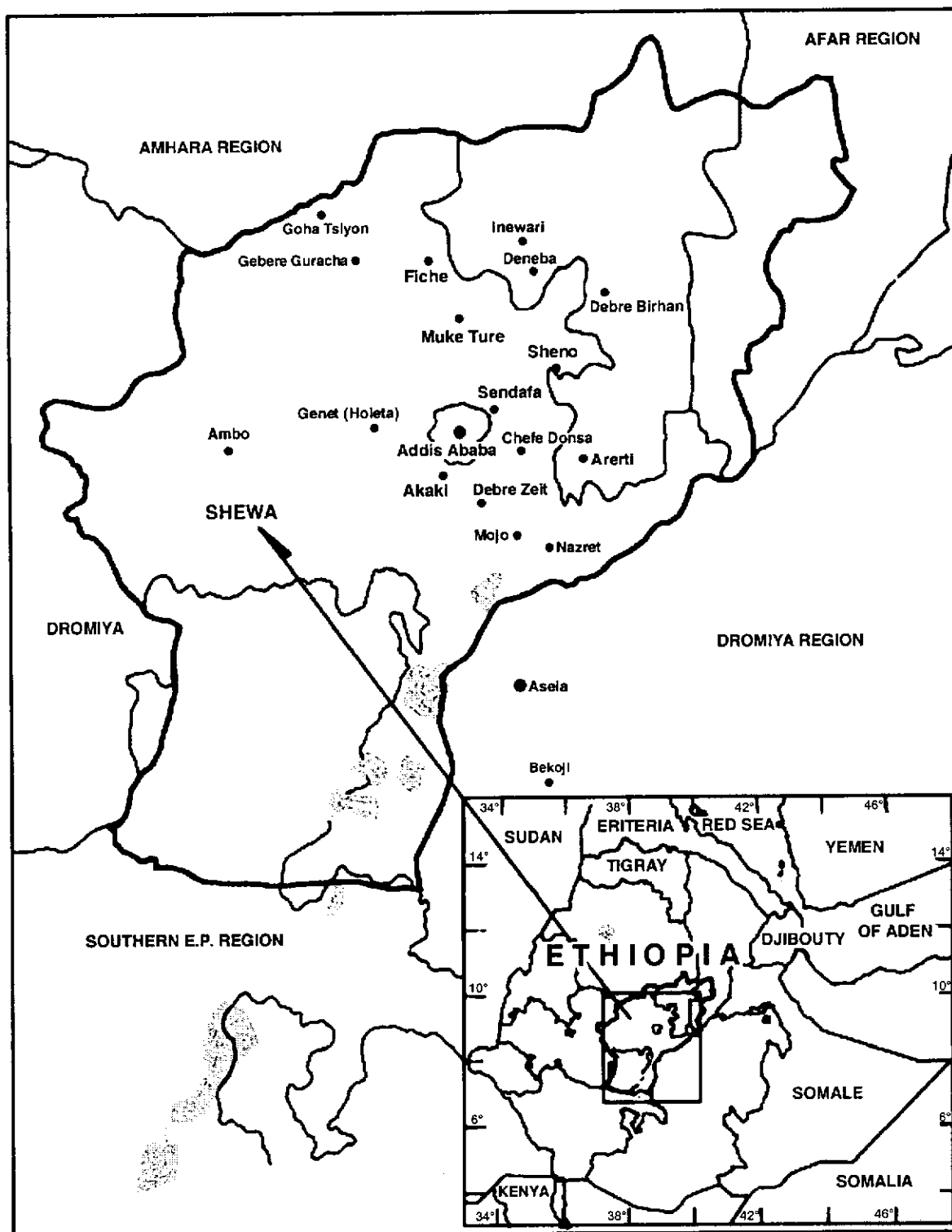


Fig. 1 - Map of Ethiopia showing the regions surveyed for chickpea and lentil virus diseases. The rectangle in the center of the general map was enlarged to show the locations covered during the survey.

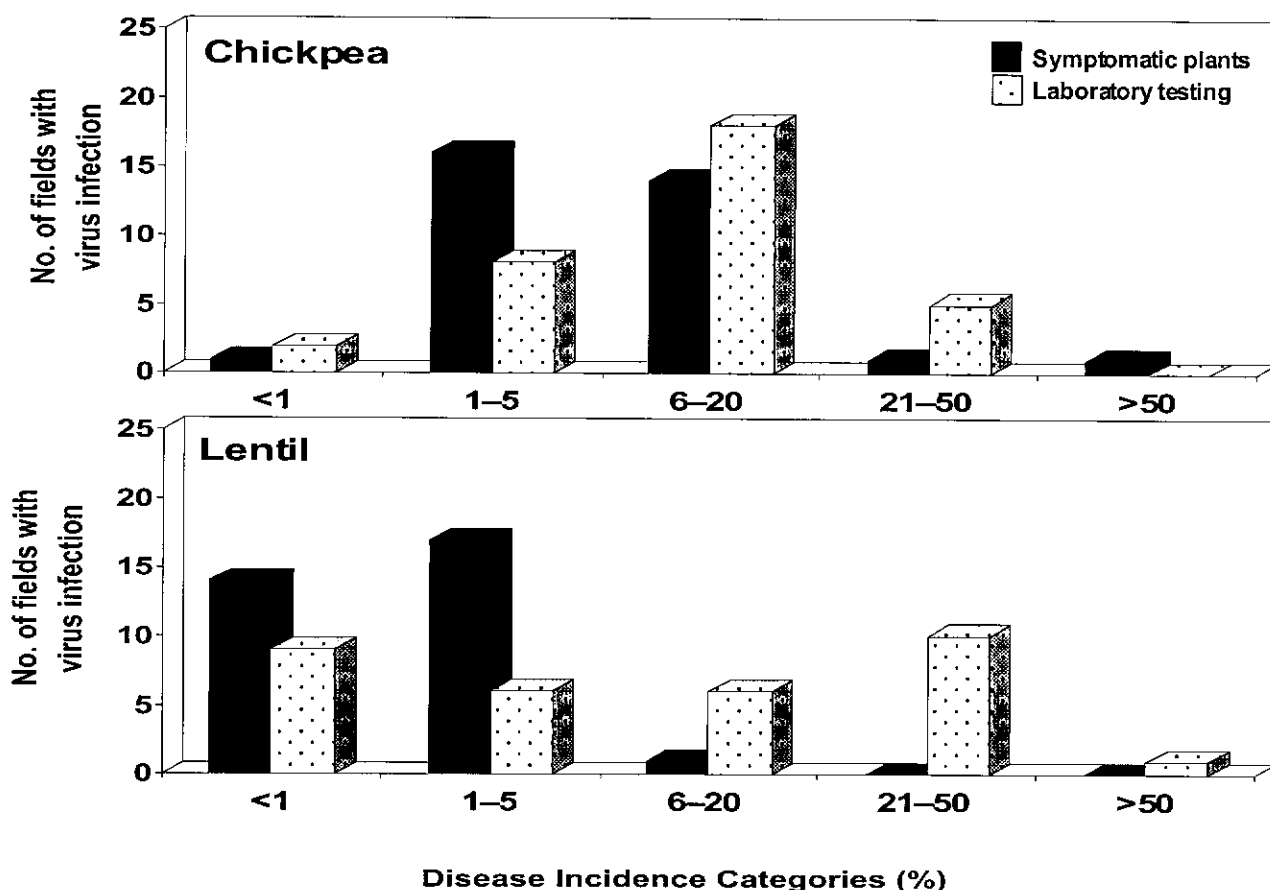


Fig. 2 - Comparison of the virus infection incidence in chickpea and lentil fields based on the presence of symptomatic plants observed in the field and on the results from laboratory testing of randomly collected samples during the survey conducted in Ethiopia, November 14-23, 1998.

in a chickpea field 41.3% with beet western yellows polerovirus (BWYV) at Akaki.

Laboratory testing of symptomatic samples from lentil fields showed that both the luteoviruses and PSbMV were common in lentil (Table I), but that only the luteoviruses were common in chickpea (Table II). BYMV and PEMV each infected one symptomatic lentil sample, and FBNYV infected two symptomatic chickpea samples.

When comparing virus occurrence in adjacent lentil and chickpea fields at 15 locations (Table III), it was found that, with the exception of two fields, the luteovirus BWYV was more common in chickpea than in lentil fields. In addition, PSbMV and FBNYV were only detected in lentil, not in chickpea fields.

Screening with specific monoclonals of 171

chickpea samples (from 14 locations) and 68 lentil samples (from 5 locations) that had given a positive reaction with the broad-spectrum legume luteovirus monoclonal antibody (5G4) showed that BWYV was the main luteovirus naturally infecting chickpea in Ethiopia, whereas on lentil BWYV and SbDV were about equally important (Table IV).

## Discussion

Beet western yellows polerovirus (BWYV) was the most commonly encountered virus in chickpea and PSbMV in lentil fields in Ethiopia. BWYV was reported earlier from Ethiopia (Hulluka and Tadesse, 1994), but identification was based on its reaction to a rabbit polyclonal antiserum. Such an antiserum is known to have a wide cross-

TABLE I. - Results of laboratory tests on lentil samples collected at random and with symptoms suggestive of virus infection from 32 fields in Shewa province of Ethiopia during November 14-23, 1998.

Location	Sample collection method	No. of fields surveyed	No. of samples tested	No. of samples found positive to (a)							Total incidence (b) (%)
				Luteo-viruses	FBNYV	PSbMV	BBSV	BYMV	PEMV	CMV	
Debre Zeit	Random	1	120	50	2	0	0	0	0	0	43.3
	Symptoms	1	45	45	0	0	0	0	0	0	
Debre Zeit - Chefe Donsa	Random	2	225	2	0	27	0	0	0	0	12.9
	Symptoms	2	80	30	1	0	0	0	0	0	
Sendafa - Chefe Donsa	Random	3	465	2	0	100	0	0	0	0	21.9
	Symptoms	3	53	6	0	8	0	0	0	0	
Akaki - Kilinto	Random	1	120	38	0	0	0	0	0	0	31.7
	Symptoms	1	23	21	0	0	0	0	0	0	
Arerti - Mojo	Random	1	90	0	0	0	0	0	0	0	0.0
	Symptoms	1	30	0	0	0	0	0	0	0	
Addis - Welkite	Random	2	255	0	0	36	0	0	0	0	14.1
	Symptoms	2	46	4	1	4	0	0	1	0	
Addis - Debre Birhan	Random	6	975	8	0	104	0	0	0	0	11.5
	Symptoms	6	138	32	0	12	0	0	0	0	
Muketuri - Deneba	Random	5	900	12	19	13	1	1	0	2	5.3
	Symptoms	5	150	2	19	1	0	0	0	0	
Muketuri - Fiche	Random	4	540	0	0	99	0	0	0	0	18.3
	Symptoms	4	100	0	3	17	0	1	0	0	
Gebre Guracha - Goha Tsiyon	Random	1	120	0	1	0	0	0	0	0	0.8
Deneba - Inewari	Random	3	500	6	0	82	0	0	0	0	17.6
	Symptoms	3	72	6	5	6	0	0	0	0	
Holetta - Ambo	Random	3	360	0	0	0	0	0	0	0	0.0
	Symptoms	3	20	0	0	0	0	0	0	0	
Total	Random	32	4670	118	22	461	1	1	0	2	13.0
	Symptoms	32	757	146	29	83	0	1	1	0	

(a) All samples were negative to AMV, CCDV, BBMV, BBWV and BBTMV.

AMV = Alfalfa mosaic alfamovirus  
 BBMV = Broad bean mottle bromovirus  
 BBSV = Broad bean stain comovirus  
 BBTMV = Broad bean true comovirus  
 BBWV = Broad bean wilt fabavirus  
 BYMV = Bean yellow mosaic potyvirus

PSbMV = Pea seed-borne mosaic potyvirus  
 PEMV = Pea enation mosaic virus  
 CMV = Cucumber mosaic cucumovirus  
 CCDV = Chickpea chlorotic dwarf geminivirus  
 FBNYV = Faba bean necrotic yellows nanovirus  
 Luteoviruses = For this test, a broad spectrum monoclonal antibody (5G4) which reacts with a number of legume luteoviruses was used

(b) Total incidence was calculated only from samples collected at random.

TABLE II. - Results of laboratory tests on chickpea samples collected at random and with symptoms suggestive of virus infection from 33 fields in Shewa province of Ethiopia during November 14-23, 1998.

Location	Sample collection method	No. of fields surveyed	No. of samples tested	Number of samples found positive to (a)			Total incidence (b) (%)
				Luteoviruses	FBNYV	BBWV	
Debre Zeit	Random	2	130	16	0	0	12.3
	Symptoms	2	37	32	0	0	
Debre Zeit – Mojo	Random	2	220	15	0	3	8.2
	Symptoms	2	40	35	0	0	
Debre Zeit – Akaki	Random	2	190	58	0	0	30.5
	Symptoms	2	50	50	0	0	
Debre Zeit – Chefe Donsa	Random	3	210	29	0	0	13.8
	Symptoms	3	51	47	1	0	
Sendafa – Chefe Donsa	Random	1	80	5	0	0	6.3
	Symptoms	1	20	20		0	
Akaki – Kilinto	Random	2	160	53	0	0	33.1
	Symptoms	2	55	54		0	
Arerti – Mojo	Random	3	330	28	0	0	8.5
	Symptoms	3	58	48	0	0	
Addis – Welkite	Random	5	510	53	2	0	10.8
	Symptoms	5	130	120	0	0	
Addis – Debre Berhan	Random	1	110	2	0	0	1.8
	Symptoms	1	12	8	0	0	
Muketuri – Deneba	Random	1	90	3	0	0	3.3
	Symptoms	1	20	15	0	0	
Muketuri – Fiche	Random	2	190	21	0	0	11.1
	Symptoms	2	80	39	0	0	
Gebre Guracha – Goha Tsiyon	Random	2	210	15	0	0	7.1
	Symptoms	2	55	24	1	0	
Deneba – Inewari	Random	2	160	5	0	0	3.1
	Symptoms	2	33	22	0	0	
Holetta – Ambo	Random	5	510	26	0	0	5.1
	Symptoms	5	95	41	0	0	
Total	Random	33	3100	329	2	3	10.8
	Symptoms	33	736	555	2	0	

(a) All samples were negative to AMV, BBMV, BBSV, PSbMV, BYMV, PEMV, CMV, CCDV, and BBTMV.

AMV = Alfalfa mosaic alfamovirus	PSbMV= Pea seed-borne mosaic potyvirus
BBMV= Broad bean mottle bromovirus	PEMV = Pea enation mosaic virus
BBSV= Broad bean stain comovirus	CMV = Cucumber mosaic cucumovirus
BBTMV= Broad bean true comovirus	CCDV = Chickpea chlorotic dwarf geminivirus
BBWV= Broad bean wilt fabavirus	FBNYV= Faba bean necrotic yellows nanovirus
BYMV = Bean yellow mosaic potyvirus	Luteoviruses= For this test, a broad spectrum monoclonal antibody (5G4) which react with a number of legume luteoviruses was used

(b) Total incidence was calculated only from samples collected at random.

TABLE III. - Comparison of virus occurrence patterns in 15 adjacent chickpea and lentil fields on the basis of laboratory testing of random samples collected from Shewa province of Ethiopia during 14–23 November, 1998.

Location	Infection with (%) (a)					
	Luteoviruses		PSbMV		FBNYV	
	Chickpea	Lentil	Chickpea	Lentil	Chickpea	Lentil
Debre Zeit	7.8	41.7	0.0	0.0	0.0	1.7
Akaki – Kilinto	25.0	31.7	0.0	0.0	0.0	0.0
Debre Zeit – Chefe Donsa	15.7	2.2	0.0	11.1	0.0	0.0
Addis – Walkite	13.6	0.0	0.0	0.0	0.0	0.0
Addis – Walkite	10.0	0.0	0.0	24.0	0.0	0.0
Addis – Debre Berhan	1.8	0.0	0.0	34.9	0.0	0.0
Sendafa – Chefe Donsa	6.3	1.9	0.0	16.2	0.0	0.0
Muketuri – Deneba	3.3	0.0	0.0	1.2	0.0	6.7
Deneba – Inewari	5.6	0.0	0.0	58.6	0.0	0.0
Holetta – Ambo	1.0	0.0	0.0	0.0	0.0	0.0
Holetta – Ambo	12.0	0.0	0.0	0.0	0.0	0.0
Holetta – Ambo	8.0	0.0	0.0	0.0	0.0	0.0
Muketuri – Fiche	5.0	0.0	0.0	33.0	0.0	0.0
Muketuri – Fiche	17.8	0.0	0.0	0.7	0.0	0.0
Muketuri – Fiche	4.0	0.0	0.0	0.0	0.0	0.8

(a) Luteoviruses = For this test, a broad spectrum monoclonal antibody (5G4) which react with a number of legume luteoviruses was used.  
 PSbMV= Pea seed-borne mosaic potyvirus.  
 FBNYV= Faba bean necrotic yellows nanovirus.

reactivity to a number of different luteoviruses (Waterhouse *et al.*, 1988). In the present study identification of BWYV was based on its reaction with the monoclonal antibody specific for BWYV, and accordingly, the occurrence of BWYV on chickpea and lentil in Ethiopia was established beyond doubt and was reported recently (Tadesse *et al.*, 1999). It can therefore be concluded that BWYV is the main cause of chickpea stunting and chlorosis in Ethiopia.

It was evident from this study that visual field inspection tended to underestimate disease incidence in both chickpea and lentil fields, but it was more in lentil than in chickpea. In lentil, 11 fields out of 32 had a virus disease incidence of 21% or higher in the laboratory tests, whereas with visual field inspection none of the lentil fields was placed in such a high disease-incidence category. Therefore, laboratory testing is essential for an accurate assessment of virus incidence in the field.

In about 15% of chickpea fields and 34.4% of lentil fields, virus disease incidence was 21% or higher. This is in agreement with an earlier report (Hulluka and Tadesse, 1994), which found that in some regions of Ethiopia, virus diseases were causing significant economic losses. More attention to the control of these diseases is therefore justified.

Faba bean necrotic yellows nanovirus (FBNYV) has been reported to infect chickpea in many countries of the world (Katul *et al.*, 1993; Horn *et al.*, 1995; Makkouk *et al.*, 1998) and it is the most harmful virus associated with chickpea stunt disease in Syria, Turkey and Lebanon (Horn *et al.*, 1995). The incidence of FBNYV on chickpea and lentil revealed in this study was rather low. However, because of the potential damage this virus can cause, it needs closer monitoring in the coming years.

It was found that in around 65% of symptomatic lentil and 24% of symptomatic chickpea

TABLE IV. - Identification of luteoviruses in chickpea and lentil samples collected from Shewa province of Ethiopia on the basis of their reaction to specific monoclonal and polyclonal antibodies.

Region	Crop	No. of samples found positive to (a)					
		Monoclonal antibodies					Polyclonal antibody
		5G4 Broad	ATCC-647 BWYV	ATCC-650 SbDV	6G4 BLRV	3 B11 BLRV or SbDV	CpLV (India)
Debre Zeit	Chickpea	3	3	0	0	0	0
	Lentil	39	20	22	3	3	0
Debre Zeit – Akaki	Chickpea	7	7	0	0	0	0
Akaki – Kilinto	Chickpea	8	8	0	0	1	0
	Lentil	21	14	13	0	2	0
Arerti – Mojo	Chickpea	14	14	0	0	0	0
Mojo – Debre Zeit	Chickpea	12	12	0	0	2	0
Debre Zeit – Chefe Donsa	Chickpea	18	18	0	0	6	0
	Lentil	1	0	0	0	1	0
Addis – Walkite	Chickpea	15	15	0	0	0	0
Addis – Debre Berhan	Chickpea	8	8	0	0	0	0
Sendafa – Chefe Donsa	Chickpea	12	12	0	0	0	0
Muketuri – Deneba	Chickpea	12	12	0	0	0	0
	Lentil	2	2	0	0	0	0
Deneba – Inewari	Chickpea	15	15	0	0	0	0
	Lentil	5	2	3	0	0	0
Holetta – Ambo	Chickpea	26	26	0	0	0	0
Muketuri – Fiche	Chickpea	10	10	0	0	0	0
Gebre Guracha – Goba Tsiyon	Chickpea	11	11	0	0	0	0
Total	Chickpea	171	171	0	0	9	0
	Lentil	68	38	38	3	6	0

(a) BLRV= Bean lead roll virus  
 BWYV= Beet western yellows virus

SbDV= Soybean dwarf virus  
 CpLV= Chickpea luteovirus

plant samples that were tested in the laboratory, no virus was detected with the antisera used. It is likely however that there are other viruses, not tested for in this study, which infect these crops. Moreover some abiotic factors (e.g., iron deficiency), produce symptoms similar to those produced by viruses and they may have caused some of the symptoms observed by field inspection (Bejiga *et al.*, 1996; Hampton *et al.*, 1998). More work needs

to be conducted in Ethiopia to clarify these possibilities.

Comparisons of virus occurrence in adjacent chickpea and lentil fields at 15 locations led to two interesting observations: (1) with the exception of two fields, the luteovirus BWYV was more common on chickpea than on lentil and, (2) PSbMV and FBNYV were detected in lentil only, not in chickpea. All these viruses are vectored by



the pea aphid *Acyrtosiphon pisum* Harris. However, during the survey, many lentil fields but none of the chickpea fields were found to be infested with *A. pisum*, and indeed it is known that this aphid prefers lentil to chickpea. Yet luteoviruses were more common in chickpea fields than in the adjacent lentil fields. This suggests that other migratory viruliferous aphid species were involved in the luteovirus infection of chickpea fields, even though they do not multiply on this crop. *A. pisum* is not the only aphid that transmits BWYV; several other species also spread this virus in a persistent manner (Duffus 1960; 1972).

At the time of the survey, few fields were approaching maturity, but a fairly large number were at the flowering stage. Virus incidence, and losses in the latter fields, would increase if environmental conditions in late November and December are favorable to the increase and spread of the aphid population.

It is not surprising that PSbMV should occur in Ethiopia, since lentil, pea and faba bean are all sown in the same season, referred to as the Meher season and is likely that the virus is spread through its aphid vector, *A. pisum*, from one crop to another (Hampton and Mink, 1975). Abraham and Albrechtsen (1998) reported the presence of PSbMV in pea in Ethiopia and the virus had previously been reported on faba bean in Ethiopia (Makkouk *et al.*, 1993), but this is the first report of PSbMV also infecting lentil. There was a large variation in PSbMV incidence in the lentil fields surveyed, from zero to 58%. As PSbMV is a seed-borne virus, the quality of the seeds used by farmers in sowing their crops is the most likely cause of this variation. The use of certified virus-free lentil seeds will greatly improve the situation as it relates to PSbMV. The role of seed-multiplication organizations in providing certified seeds to farmers will pay dividend to farmers and to the country.

Results obtained in this study indicated that on lentil BWYV and SbDV were equally important. Makkouk *et al.* (1997) reported the natural occurrence of SbDV on lentil in Syria. The present study is apparently the first to report SbDV as naturally infecting lentil in Ethiopia. Other viruses such as BBSV, BYMV, CMV and PEMV were detected in lentil, but their occurrence was rare.

Nine chickpea and one lentil sample reacted

with the monoclonal 3B11, but not with the BLRV-specific 6G4 or with the SbDV-specific monoclonals. Katul (1992) found that the monoclonal 3B11 reacts with either BLRV or SbDV. Results obtained here with Ethiopian isolates suggest that 3B11 also reacted with luteoviruses other than BLRV or SbDV.

Viruses in chickpea other than those mentioned were rarely detected. They include FBNYV (0.06%) and BBWV (0.09%). Nene *et al.* (1996) and Makkouk *et al.* (1998) reported the occurrence of these two viruses in association with chickpea in Pakistan, Syria, Turkey and Lebanon. Aphids transmit FBNYV in a persistent manner, whereas BBWV is both seed- and aphid-transmissible (Makkouk *et al.*, 1990). This is the first report of FBNYV and BBWV infecting chickpea in Ethiopia.

About 0.13% of symptomatic lentil samples collected from the surveyed areas tested positive for pea enation mosaic enamovirus (PEMV) (Table I). Lentil is known to be susceptible to PEMV and the virus is mechanically transmissible and is transmitted by aphids in a persistent manner (Bos and Makkouk, 1994). The natural occurrence of PEMV on lentil has been recorded recently in Syria (Makkouk *et al.*, 1999), but this is the first report of PEMV on lentil in Ethiopia.

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