

## RESEARCH PAPERS

# Survey of viruses affecting legume crops in the Amhara and Oromia Regions of Ethiopia

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**Summary.** Field surveys were undertaken to identify the viral diseases affecting lentil, faba bean, chickpea, pea, fenugreek and grass pea in two regions of Ethiopia. The surveys were conducted in the regions of Amhara (Gonder and Gojam administrative zones) and Oromia (Bale administrative zone) during the 2003/2004 and 2004/2005 growing seasons, respectively. The survey covered 138 randomly selected fields (48 faba bean, 10 pea, 38 grass pea, 34 chickpea, 8 lentil) in the Amhara region, and 51 legume fields (29 faba bean, 12 pea, 3 lentil, 5 fenugreek, 2 chickpea) in the Oromia region. Virus disease incidence was determined by laboratory testing of 100–200 randomly-collected samples from each field against the antisera of 12 legume viruses. Of the 189 fields surveyed, 121 and 7 had, at the time of the survey, a virus disease incidence of 1% or less and more than 6%, respectively, based on visual inspection in the field; later laboratory testing showed that the number of fields in these two categories was in fact 99 and 56, respectively. Serological tests indicated that the most important viruses in the Amhara region were *Faba bean necrotic yellows virus* (FBNYV), *Bean yellow mosaic virus* (BYMV), *Pea seed-borne mosaic virus* (PSbMV) and the luteoviruses [e.g. *Beet western yellows virus* (BWYV), *Bean leaf roll virus* (BLRV), *Soybean dwarf virus* (SbDV)]. By contrast, only FBNYV and the luteoviruses were detected in the Oromia region. Other viruses, such as *Broad bean mottle virus* (BBMV) and *Alfalfa mosaic virus* (AMV), were rarely detected in the Amhara region. This is the first report in Ethiopia of natural infection of faba bean, pea and fenugreek with SbDV, of fenugreek with BWYV, and of grass pea with BYMV, PSbMV and BWYV, and it is also the first recorded instance of BBMV infecting legume crops in Ethiopia.

**Key words:** cool-season food legumes, *Lathyrus sativus* L., *Trigonella foenum-graecum* L., virus diseases.

## Introduction

The Amhara and Oromia regions are the largest producers of pulses in Ethiopia. The major pro-

duction areas in the Amhara region are the Gojam and Gonder administrative zones (northwest Ethiopia), while in the Oromia region, the Bale administrative zone is the major production area. The contribution of the Amhara region to the total production area of pulses in Ethiopia was about 48% in the 2001–2002 cropping season (CSA, 2002). Faba bean (*Vicia faba* L.), chickpea (*Cicer*

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*arietinum* L.), field pea (*Pisum sativum* L.), grass pea (*Lathyrus sativus* L.), lentil (*Lens culinaris* Medik) and fenugreek (*Trigonella foenum-graecum* L.) are the most important cool-season food legumes grown in the Amhara region, in terms of both area and production. In the 2003–2004 season, the average yields of these crops were 1167 (faba bean), 921 (grass pea), 892 (chickpea), 849 (field pea), 783 (fenugreek) and 728 kg ha<sup>-1</sup> (lentil) (CSA, 2004). In the Oromia region, faba bean, field pea, chickpea, grass pea, lentil and fenugreek are, in that order, the most important cool-season food legumes grown. In terms of average yield in the 2003–2004 season, however, grass pea yielded most (1107 kg ha<sup>-1</sup>), followed by faba bean (1096), chickpea (896), field pea (787), fenugreek (727) and lentil (562 kg ha<sup>-1</sup>) (CSA, 2004).

Fungal and viral diseases are important factors limiting the production of food-legume crops in Ethiopia, including the Amhara and Oromia regions (Habtu and Gorfu, 1986; Amare and Beniwal, 1988). Several of the viruses found in Ethiopia are known to infect these crops worldwide, some causing serious economic losses (Cockbain, 1983; Bos *et al.*, 1988; Makkouk *et al.*, 2003). Surveys conducted between 1996 and 1997 in the faba bean growing areas in the central and northeastern parts of Ethiopia, as well as in the Arsi administrative zone in the southeast, showed that eight viruses occurred in faba bean; *Faba bean necrotic yellow virus* (FBNYV, genus *Nanovirus*, family *Nanoviridae*) was the most frequent, followed by the luteoviruses (e.g. *Beet western yellows virus* [BWYV, genus *Polerovirus*, family *Luteoviridae*] and *Bean leaf roll virus* [BLRV, family *Luteoviridae*]) (Abraham *et al.*, 2000). Another survey of lentil and chickpea viruses, conducted in central Ethiopia in 1998, showed that nine viruses were present in lentil and three in chickpea (Tadesse *et al.*, 1999). In chickpea fields the luteoviruses were the most commonly identified viruses; in lentil fields, *Pea seed-borne mosaic virus* (PSbMV, genus *Potyvirus*, family *Potyviridae*) was the most common virus, while the luteoviruses were in second place. In a survey of viruses of grass pea in Ethiopia, only FBNYV was detected in symptomatic (stunted and yellowed) samples collected from a small number of locations in the administrative zones of Shewa, Arsi, Wello, Gojam and Gonder (Abraham and Lencho, 2000).

No systematic survey of viruses infecting food legumes in the administrative zones of the Amhara and Oromia regions has yet been carried out. The objective of this work was therefore to identify and document the viral diseases of legume crops in these zones, in order to complement earlier surveys (Tadesse *et al.*, 1999; Abraham *et al.*, 2000) in Ethiopia.

## Materials and methods

### Field visits, sample collection and virus disease incidence

#### *Amhara region (Gonder and Gojam zones)*

Two consecutive surveys were conducted in the Amhara region. Survey I, was conducted from September 1 to 9, 2003. In this survey a total of 540 samples (468 faba bean, 72 pea) with symptoms suggestive of virus infection (yellowing, stunting, or mosaic/mottling), and 11600 random samples (9600 faba bean, 2000 pea) were collected from 58 fields (48 faba bean, 10 pea). Survey II, conducted from December 26, 2003 to January 4, 2004. Here a total of 1130 symptomatic samples (670 grass pea, 380 chickpea, 80 lentil), and 3740 random samples (2050 grass pea, 1450 chickpea, 240 lentil) were collected from 80 fields (38 grass pea, 34 chickpea, 8 lentil).

#### *Oromia region (Bale zone)*

One survey was carried out in the Oromia region (Bale zone) from November 18 to 28, 2004. In this survey, a total of 451 samples with symptoms suggestive of virus infection (202 faba bean, 83 pea, 49 lentil, 84 fenugreek, 33 chickpea), and 9310 random samples (5610 faba bean, 2260 pea, 570 lentil, 550 fenugreek, 320 chickpea) were collected from 51 fields (29 faba bean, 12 pea, 3 lentil, 5 fenugreek, 2 chickpea).

In all the above surveys, fields were selected randomly when crops were at the flowering-pod setting stage, and the distance between fields sampled was determined on a daily basis. The areas covered in this survey are shown in Fig. 1. Each field surveyed was evaluated using a standard method. Virus disease incidence in each field was determined on the basis of visual symptoms, and the number of infected plants at different, randomly chosen, locations in the field was counted; this information was then used to calculate

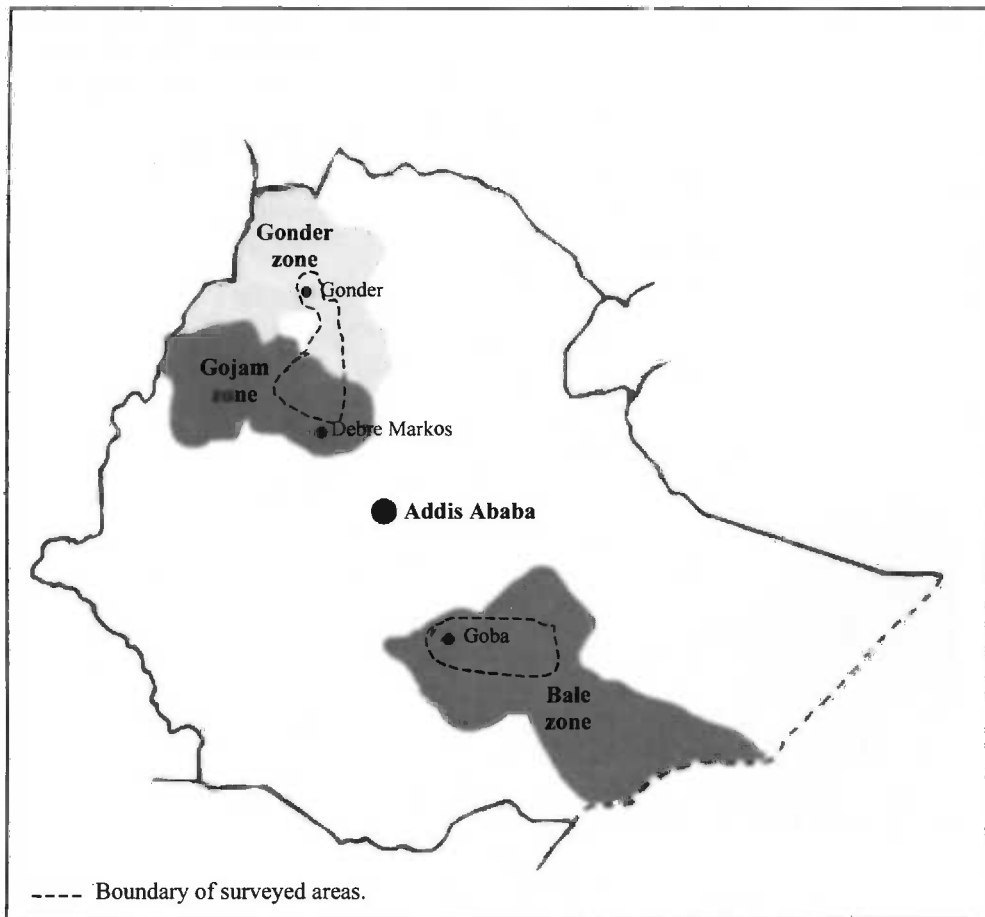


Fig. 1. Map of Ethiopia showing the areas surveyed, in three administrative zones, during the 2003–2004 and 2004–2005 growing seasons.

the percentage of infected plants (virus incidence) per field. From each field, two types of samples were collected: one from 10–25 symptomatic plants, and one from 100–200 randomly collected plants irrespective of whether they showed symptoms of viral disease. All samples were placed in labelled plastic bags and taken to the laboratory for testing. Most samples were blotted on nitrocellulose membranes on the day that they were collected. Testing for key viruses was conducted in the Pathology Laboratories of the Adet and Sinana Agricultural Research Centres in the Amhara and Oromia respectively. Tests for viruses were also carried out at the Virology Laboratory of the Plant Protection Research Centre (PPRC), Ambo, Ethiopia, and at the Virology

Laboratory of the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

#### Laboratory tests

All samples were tested for the presence of viruses using the tissue-blot immunoassay (TBIA) technique (Makkouk and Comeau, 1994; Makkouk and Kumari, 1996); a battery of polyclonal and monoclonal antibodies (McAbs) were used. ICARDA's Virology Laboratory provided rabbit polyclonal antisera for the following: *Alfalfa mosaic virus* (AMV, genus *Alfamovirus*, family *Bromoviridae*), *Bean yellow mosaic virus* (BYMV, genus *Potyvirus*, family *Potyviridae*), *Broad bean mottle virus* (BBMV, genus *Bromovirus*, family

*Bromoviridae*), *Broad bean stain virus* (BBSV, genus *Comovirus*, family *Comoviridae*), *Broad bean true mosaic virus* (BBTMV, genus *Comovirus*, family *Comoviridae*), *Broad bean wilt virus* (BBWV, genus *Fabavirus*, family *Comoviridae*), *Chickpea chlorotic dwarf virus* (CpCDV, genus *Mastrevirus*, family *Geminiviridae*), *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family *Bromoviridae*) and PSbMV. A McAb to detect FBNYV (3-2E9) was provided by A. Franz (Franz *et al.*, 1996), and a broad-spectrum legume-luteovirus McAb (5G4) was provided by L. Katul (Katul, 1992), BBA, Braunschweig, Germany.

To identify the individual luteoviruses affecting legume crops in the areas studied, those infected samples that gave a positive reaction to the broad-spectrum McAb 5G4 were further tested against three specific McAbs: one for BWYV (from Agdia Lot No. 0499, USA), one for BLRV (4B10; Katul, 1992) and one for *Soybean dwarf virus* (SbDV, family *Luteoviridae*) (ATCC PVAS-650).

## Results

### Virus disease incidence as determined by field observation

#### Amhara region (Gonder and Gojam zones)

Survey I - The virus-like disease symptoms most commonly observed in faba bean and pea fields were mottling/mosaic, chlorosis, stunting, yellowing, and reddening of the leaves. The virus disease incidence assessed by a visual search for virus-like symptoms was less than 1% of all faba bean and pea fields surveyed (Table 1).

Survey II - The most commonly observed virus disease symptoms in grass pea, chickpea and lentil fields were mosaic/mottling, stunting, chlorosis, yellowing and reddening of leaves, and leaf and stem necrosis. Based on the symptoms observed in the field, 75% (60 fields) of all fields (27 grass pea, 28 chickpea, 5 lentil) had a virus incidence of 1–5% (Table 1). Seven fields had a virus incidence of 6–20% (one grass pea, 3 chickpea, 3 lentil) (Table 1).

Table 1. Virus incidence in legume crops, as determined from symptomatic samples in the field (S) and from laboratory testing of randomly collected samples (L), during surveys conducted in the Amhara (Gonder and Gojam zones) and Oromia (Bale zone) regions, Ethiopia, in 2003 and 2004.

Region/zone/Crop	No. of fields surveyed	Number of fields with virus incidence (%) of									
		< 1		1–5		6–20		21–50		> 50	
		S	L	S	L	S	L	S	L	S	L
Amhara region (Gonder and Gojam zones)											
Survey I											
Faba bean	48	48	48	0	0	0	0	0	0	0	0
Pea	10	10	10	0	0	0	0	0	0	0	0
Survey II											
Grass pea	38	10	18	27	8	1	7	0	4	0	1
Chickpea	34	3	5	28	6	3	16	0	7	0	0
Lentil	8	0	0	5	0	3	1	0	2	0	5
Oromia region (Bale zone)											
Faba bean	29	29	6	0	16	0	7	0	0	0	0
Pea	12	12	7	0	2	0	2	0	1	0	0
Lentil	3	3	2	0	0	0	1	0	0	0	0
Fenugreek	5	4	2	1	1	0	1	0	1	0	0
Chickpea	2	2	1	0	1	0	0	0	0	0	0

*Oromia region (Bale zone)*

Virus disease incidence, based on virus-like symptoms detected by visual inspection, was less than 1% in all fields surveyed, except in the case of one fenugreek field near Goro, which had an incidence of around 5% (Table 1).

**Insect vectors***Amhara region (Gonder and Gojam zones)*

Survey I - In the field inspections during the survey, no aphids were observed in 28 of the faba bean and 10 of the pea fields surveyed. In 17 faba bean fields, aphids (*Acyrtosiphon pisum*, *Aphis craccivora* and/or *A. fabae*) were present at a low levels; and large aphid populations occurred in only three faba bean fields.

Survey II - High to low levels of aphid infestation were recorded in 36 out of 38 grass pea fields surveyed. In lentil, aphids were recorded in 4 out of 8 fields, while no aphid vectors were encountered in any of the chickpea fields surveyed.

*Oromia region (Bale zone)*

In field inspections during the survey, no aphids were found in 24 of the fields surveyed (14 faba bean, 3 pea, 3 lentil, 2 fenugreek, 2 chickpea). Aphids (*A. pisum*, *A. craccivora* and/or *A. fabae*) were present in low levels in 23 fields (15 faba bean, 5 pea, 3 fenugreek), and at high levels in 4 pea fields (mainly *A. pisum*).

**Virus identification and incidence as determined by laboratory testing***Amhara region (Gonder and Gojam zones)*

Survey I - Laboratory testing of the 9600 randomly collected faba bean samples indicated that FBNYV was the most common virus, with an overall mean incidence of 0.28%, followed by BYMV (0.25%) and the luteoviruses (0.19%). No viruses were detected at all in the 2000 pea samples randomly collected from 10 fields (Table 2). Testing of faba bean symptomatic samples showed that only nine out of the 468 samples tested reacted with the McAb 5G4 (Table 2). No virus was detected in any symptomatic pea samples.

Survey II - Laboratory testing of 2050 randomly collected grass pea samples indicated that the overall incidence of viral infection was 9.6%. The luteoviruses were the most common, with an overall mean incidence of 4.5%, followed by PSbMV

(3.9%), FBNYV (1.0%) and BYMV (0.1%). Viral disease incidence in chickpea fields, computed on the basis of randomly collected samples, was 12.3% (Table 3); the luteoviruses were the most common (8.9%), followed by PSbMV (2.4%). The incidence of BBMV, FBNYV and AMV was extremely low (less than 1%). By contrast, in lentil fields, virus disease incidence as determined from the randomly collected samples was high (70.8%) (Table 3). Here PSbMV was the most common (52.1%), followed by the luteoviruses (10.8%), BBMV (7.5%) and BYMV (0.4%).

*Oromia region (Bale zone)*

Laboratory testing of the 9310 randomly collected legume crop samples showed that the luteoviruses occurred most frequently, with an overall mean incidence of 3.7%, followed by FBNYV (0.7%) (Table 4). Of the 451 symptomatic samples, only 89 reacted with the McAb 5G4 (luteoviruses) and only 21 with FBNYV (Table 4). Virus incidence as determined from the randomly collected samples was 9.6%, 5.8%, 3.7%, 2.1% and 1.9% in fenugreek, pea, faba bean, lentil and chickpea fields, respectively (Table 4).

**Identification of specific luteoviruses**

A total of 809 samples (64 faba bean, 122 grass pea, 332 lentil, 253 chickpea, 11 pea, 27 fenugreek) reacted positive to the broad-spectrum legume-luteovirus McAb 5G4. When these samples were retested with specific McAbs, three luteoviruses (BWYV, BLRV and SbDV) were identified (Table 5). An additional 114 grass pea, 218 chickpea and 320 lentil samples from the Amhara region (*Survey II*) reacted with the broad-spectrum McAb 5G4, but not with any of the BLRV-, BWYV- and SbDV-specific McAbs used.

**Comparison of field-observed virus incidence with laboratory test results**

Table 1 shows virus incidence based on (i) the percentage of plants visually assessed to have virus-like symptoms in the field, and (ii) laboratory testing of randomly collected samples. In Survey I in the Amhara region, the virus incidence assessments based on visual symptoms were similar to those obtained by testing randomly collected samples. By contrast, in Survey II in the Amhara region and in the survey conducted in the Bale zone

Table 2. Results of laboratory tests conducted on faba bean and pea samples randomly collected and with symptoms suggestive of virus infection from 48 faba bean and 10 pea fields in the Amhara region (Gonder and Gojam zones), Ethiopia, during the period September 1–9, 2003 (Survey I). Viral identification was based on serological reactions (TBIA). Figures in brackets represent virus incidence (%).

Crop	Route	No. of fields surveyed	Sample collection method	No. of samples tested	No. of samples found positive to <sup>a</sup>			Average incidence (%) <sup>b</sup>
					FBNYV	BYMV	5G4	
Faba bean	Adet-Mota-Debre Work	9	Symptoms	68	0	0	3	1.44
			Random	1800	10	12	4	
	Bahir Dar-Bure-Denbecha	9	Symptoms	49	0	0	2	0.27
			Random	1800	1	2	2	
	Addis Zemen-Ebnet	7	Symptoms	98	0	0	1	0.57
			Random	1400	4	1	3	
	Debre Tabor-Nifas Mawcha-Deber Zebit	9	Symptoms	159	0	0	3	0.55
			Random	1800	1	4	5	
	Addis Zemen-Gonder-Dabat	14	Symptoms	94	0	0	0	0.71
			Random	2800	11	5	4	
Subtotal (Faba bean)		48	Symptoms	468	0	0	9	0.72
			Random	9600	27 (0.28)	24 (0.25)	18 (0.19)	
Pea	Adet-Mota-Debre Work	7	Symptoms	60	0	0	0	0
			Random	1400	0	0	0	
	Debre Tabor-Nifas Mawcha-Deber Zebit	2	Symptoms	9	0	0	0	0
			Random	400	0	0	0	
	Addis Zemen-Gonder-Dabat	1	Symptoms	3	0	0	0	0
			Random	200	0	0	0	
Subtotal (Pea)		10	Symptoms	72	0	0	0	0
			Random	2000	0	0	0	
Total		58	Symptoms	540	0	0	9	0.60
			Random	11600	27 (0.2)	24 (0.2)	18 (0.15)	

<sup>a</sup> All samples were negative to CpCDV, BBSV, PSbMV, BBMV, BBWV, BBTMV, AMV and CMV.

Virus acronyms used: FBNYV, *Faba bean necrotic yellows virus*; BYMV, *Bean yellow mosaic virus*; CpCDV, *Chickpea chlorotic dwarf virus*; BBSV, *Broad bean stain virus*; PSbMV, *Pea seed-borne mosaic virus*; BBMV, *Broad bean mottle virus*; BBWV, *Broad bean wilt virus*; BBTMV, *Broad bean true mosaic virus*; AMV, *Alfalfa mosaic virus*; CMV, *Cucumber mosaic virus*; 5G4, A broad spectrum monoclonal reacting with all legume luteoviruses.

<sup>b</sup> Total incidence was calculated only from samples collected at random.

of the Oromia region, visual assessment grossly underestimated virus incidence. In the Amhara region (Survey II), for example, only 7 fields were placed in the 'greater than 6% incidence' category by visual assessment, whereas laboratory testing of randomly collected samples indicated that 43 fields fell into this category.

## Discussion

Visual field assessments and laboratory tests revealed considerable differences in virus incidence between the two areas surveyed, as well as between crops. For example, in laboratory tests on randomly collected samples, no viruses at all were detected on pea samples from the Amhara region, but a vi-

Table 3. Results of laboratory tests conducted on grass pea, chickpea and lentil samples randomly collected or with symptoms suggestive of virus infection from 38 grass pea, 34 chickpea and 8 lentil fields in the Amhara region (Gonder and Gojam zones), Ethiopia, between December 26, 2003 and January 4, 2004 (Survey II). Viral identification was based on serological reactions (TBIA). Figures in brackets represent virus incidence (%).

Crop/Route	No. of fields surveyed	Sample collection method	No. of samples collected	Number of samples found positive to <sup>a</sup>						Average incidence (%) <sup>b</sup>
				5G4	FBNYV	PSbMV	BBMV	BYMV	AMV	
<b>Grass pea</b>										
Dejen-Bichena	3	Symptoms	100	8	2	0	0	0	0	12.7
		Random	300	32	5	1	0	0	0	
Bichena-Mota	6	Symptoms	140	9	44	0	0	0	0	11.2
		Random	600	56	1	10	0	0	0	
Mota-Bahir Dar	2	Symptoms	50	1	0	0	0	0	0	0.5
		Random	200	0	1	0	0	0	0	
Addis Zemen-Gonder	4	Symptoms	100	8	2	28	0	0	0	13.3
		Random	120	1	3	12	0	0	0	
Gonder-Koladiba	4	Symptoms	80	1	0	1	0	0	0	11.2
		Random	260	2	8	19	0	0	0	
Ikle-Gonder	2	Symptoms	30	0	0	0	0	0	0	1.7
		Random	60	0	0	1	0	0	0	
Addis Zemen-Ibenat	2	Symptoms	20	0	0	10	0	0	0	55.0
		Random	60	0	2	31	0	0	0	
Denbecha-Jiga	3	Symptoms	30	2	0	0	0	0	0	1.1
		Random	90	0	1	0	0	0	0	
Bahir Dar-Adet	6	Symptoms	60	0	0	1	0	0	0	2.8
		Random	180	0	0	5	0	0	0	
Woreta-Woldya	6	Symptoms	60	0	0	2	0	0	0	2.8
		Random	180	2	0	1	0	2	0	
Subtotal (Grass pea)	38	Symptoms	670	29 (4.3)	48 (7.2)	42 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	9.6
		Random	2050	93 (4.5)	21 (1.0)	80 (3.9)	0 (0.0)	2 (0.1)	0 (0.0)	
<b>Chickpea</b>										
Dejen-Bichena	3	Symptoms	70	24	0	0	0	0	0	16.0
		Random	200	31	1	0	0	0	0	
Bichena-Mota	4	Symptoms	40	16	0	0	0	0	0	8.7
		Random	300	25	0	0	0	0	1	
Mota-Bahir Dar	1	Symptoms	10	4	0	0	0	0	0	11.0
		Random	100	7	4	0	0	0	0	
Addis Zemen-Gonder	4	Symptoms	30	13	0	0	0	0	0	14.2
		Random	120	17	0	0	0	0	0	
Gonder-Koladiba	4	Symptoms	60	23	0	0	0	0	0	10.5
		Random	190	9	0	8	3	0	0	
Ikle-Gonder	3	Symptoms	20	3	0	0	0	0	0	16.7
		Random	90	15	0	0	0	0	0	
Addis Zemen-Ibenat	6	Symptoms	60	17	0	0	0	0	0	13.3
		Random	180	23	1	0	0	0	0	

(continued on the next page)

Table 3. (continued)

Crop/Route	No. of fields surveyed	Sample collection method	No. of samples collected	Number of samples found positive to <sup>a</sup>						Average incidence (%) <sup>b</sup>
				5G4	FBNYV	PSbMV	BBMV	BYMV	AMV	
Denbecha-Jiga	4	Symptoms	40	10	0	0	0	0	0	5.8
		Random	120	2	1	0	4	0	0	
Bahir Dar-Adet	4	Symptoms	40	5	0	0	0	0	0	22.5
		Random	120	0	0	27	0	0	0	
Woreta-Woldya	1	Symptoms	10	4	0	0	0	0	0	0.0
		Random	30	0	0	0	0	0	0	
Subtotal (Chickpea)	34	Symptoms	380	119 (31.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	12.3
		Random	1450	129 (8.9)	7 (0.5)	35 (2.4)	7 (0.5)	0 (0.0)	1 (0.1)	
<b>Lentil</b>										
Debark-Gonder (Subtotal)	8	Symptoms	80	9 (11.3)	6 (7.5)	36 (45.0)	1 (1.3)	0 (0.0)	0 (0.0)	70.8
		Random	240	26 (10.8)	0 (0.0)	125 (52.1)	18 (7.5)	1 (0.4)	0 (0.0)	
Overall total	80	Symptoms	1130	157 (13.9)	54 (4.8)	78 (6.9)	1 (0.1)	0 (0.0)	0 (0.0)	14.6
		Random	3740	248 (6.6)	28 (0.8)	240 (6.4)	25 (0.7)	3 (0.1)	1 (0.03)	

<sup>a</sup> All samples were negative to CpCDV, BBSV, BBWV, BBTMV and CMV (see Table 2).

<sup>b</sup> Total incidence was calculated only from samples collected at random.

Table 4. Results of laboratory tests conducted on food legume samples, randomly collected or with symptoms suggestive of virus infection, from 53 fields in the Oromia region (Bale zone), Ethiopia, between November 18 and 28, 2004. Viral identification was based on serological reactions (TBIA). Figures in brackets represent virus incidence (%).

Crop/Route	No. of fields surveyed	Sample collection method	No. of samples tested	No. of samples found positive to <sup>a</sup>		Average incidence (%) <sup>b</sup>
				5G4	FBNYV	
<b>Faba bean</b>						
Dinsho-Robe-Goba	8	Symptoms	69	10	4	2.7
		Random	1550	33	7	
Gazera-Ali-Agarfa-Robe	6	Symptoms	56	18	0	8.2
		Random	1140	80	13	
Sinana Station	2	Symptoms	8	0	0	1.3
		Random	400	0	5	
Sinana1-Alemgena-Hisu-Robe	4	Symptoms	24	2	0	2.5
		Random	750	7	12	
Sof Omar-Goro-Sinana	4	Symptoms	12	1	4	5.4
		Random	770	34	8	
Gasera-Jara-Ginir	5	Symptoms	33	3	0	1.0
		Random	1000	2	8	
Subtotal (Faba bean)	29	Symptoms	202	34	8	3.7
		Random	5610	156 (2.8)	53 (0.94)	

(continued on the next page)



Table 4. (continued)

Crop/Route	No. of fields surveyed	Sample collection method	No. of samples tested	No. of samples found positive to <sup>a</sup>		Average incidence (%) <sup>b</sup>
				5G4	FBNYV	
<b>Pea</b>						
Dinsho-Robe-Goba	2	Symptoms	11	0	0	
		Random	360	13	0	3.6
Gazera-Ali-Agarfa-Robe	2	Symptoms	9	2	0	
		Random	400	3	0	0.8
Sinanal-Alemgena-Hisu	2	Symptoms	13	3	0	
		Random	370	42	0	11.4
Sinana Station	1	Symptoms	1	0	0	
		Random	170	68	0	40.0
Sof Omar-Goro-Sinana	2	Symptoms	17	2	0	
		Random	400	0	3	0.8
Gasera-Jara-Ginir	3	Symptoms	32	4	0	
		Random	560	0	1	0.2
Subtotal (Pea)	12	Symptoms	83	11	0	
		Random	2260	126 (5.6)	4 (0.2)	5.8
<b>Lentil</b>						
Gasera-Ali-Agarfa-Robe	1	Symptoms	- <sup>c</sup>	-	-	
		Random	180	0	0	0.0
Sinana Station	1	Symptoms	13	9	0	
		Random	200	0	0	0.0
Sof Omar-Goro	1	Symptoms	36	3	11	
		Random	190	4	8	6.3
Subtotal (Lentil)	3	Symptoms	49	12	11	
		Random	570	4 (0.7)	8 (1.4)	2.1
<b>Fenugreek</b>						
Gasera-Ali-Agarfa-Robe	1	Symptoms	21	18	0	
		Random	-	-	-	-
Alemgena-Hisu	1	Symptoms	20	5	0	
		Random	100	4	0	4.0
Sinana Station	1	Symptoms	6	4	0	
		Random	130	49	0	37.7
Jara-Ginir-Goro	2	Symptoms	37	0	2	
		Random	320	0	0	0.0
Subtotal (Fenugreek)	5	Symptoms	84	27	2	
		Random	550	53 (9.6)	0 (0.0)	9.6
<b>Chickpea</b>						
Sof Omar-Goro (Subtotal)	2	Symptoms	33	5	0	
		Random	320	3 (0.93)	3 (0.93)	1.9
Total (Legume)	51	Symptoms	451	89	21	
		Random	9310	342 (3.7)	68 (0.7)	4.4

<sup>a</sup> All samples were negative to CpCDV, BBSV, BYMV, BBMV, BBTMV, BBWV, AMV and CMV (see Table 2).

<sup>b</sup> Total incidence was calculated only from samples collected at random.

<sup>c</sup> No samples collected.

Table 5. Identification of luteoviruses in legume samples collected from Amhara (Gonder and Gojam zones) and Oromia (Bale zone) regions, Ethiopia, on the basis of their reaction to specific monoclonal antibodies.

Region/Crop	No. of samples found positive to monoclonal antibodies against <sup>a</sup>				No. of unidentified luteovirus samples
	5G4	BWYV (Agdia Lot No. 0499)	BLRV (4B10)	SbDV (ATCC PVAS 650)	
Amhara region (Gonder and Gojam zones)					
Survey I					
Faba bean	30	18	5	3	4
Survey II					
Grass pea	122	8	0	0	114
Chickpea	248	30	0	0	218
Lentil	320	0	0	0	320
Oromia region (Bale zone)					
Faba bean	34	9	0	25	0
Pea	11	0	0	11	0
Lentil	12	12	0	0	0
Fenugreek	27	14	0	13	0
Chickpea	5	5	0	0	0
Total	809	96	5	52	656

<sup>a</sup> BLRV, *Bean leaf roll virus*, SbDV, *Soybean dwarf virus*, BWYV, *Beet western yellows virus*, 5G4, A broad spectrum monoclonal reacting with all legume luteoviruses.

rus incidence of 5.8% was calculated for pea samples from the Bale zone, Oromia region. By contrast, the virus incidence in lentil fields in the Bale zone was 2.1% and the only viruses detected were FBNYV and luteoviruses, whereas in lentil fields in the Amhara region (Survey II) the virus incidence was 70.8% and five viruses were detected (mainly PSbMV, 52.1% incidence).

Tadesse *et al.* (1999) reported that PSbMV was the most common virus detected on lentil crops in central Ethiopia. In addition, Abraham and Makkouk (2002) reported that PSbMV was also detected in 31.1% of lentil seed samples collected in 1998 and 1999 from farmers' seed lots in central (Shewa and Arsi zones) and northern Ethiopia (Gojam, Gonder and Wello zones). In our study, PSbMV was detected in 52.1% of randomly collected lentil samples from the Amhara region. In previous studies, crop yield losses due to this virus ranged from 2.7 to 61%, depending on the lentil genotype (Kumari and Makkouk, 1995). However, the yield losses caused by PSbMV should be

scrutinized more closely, since this virus is seed-borne in lentil and other legume crops (Makkouk *et al.*, 1993), and is transmitted by aphids in a non-persistent manner (Gonzalez and Hagedorn, 1971; Aapola and Mink, 1973). *Aphis craccivora*, *A. fabae* and *A. pisum* are known vectors of this virus (Makkouk *et al.*, 1993), and they were detected in large numbers in some of the fields surveyed in this study.

It should also be mentioned here that the PSbMV transmission rates through lentil seeds may vary from 6% (Makkouk *et al.*, 1993) to 44% (Hampton and Muehlbauer, 1977). The high incidence of PSbMV in the lentil fields surveyed suggested that farmers very probably sowed infected seeds. Accordingly, the use of healthy seeds for planting should be promoted as an important part of the production package for lentil in Ethiopia.

A total of 652 samples (114 grass pea, 218 chickpea, 320 lentil) reacted with the legume broad-spectrum McAb 5G4 but not with any of the

specific antibodies used in this study. This indicates that luteoviruses other than BWYV, BLRV and SbDV infecting legume crops are present in Ethiopia.

An earlier study (Abraham and Lencho, 2000) using transmission tests, serology and electron microscopy reported that FBNYV occurred in grass pea samples from plants with severe stunting and yellowing. No other viruses causing these symptoms were found to be associated with grass pea in that study. In the present investigation, however, four viruses (the luteoviruses, PSbMV, FBNYV and BYMV, in order of importance), were detected in grass pea in the Amhara region. Moreover, the incidence of the luteoviruses and PSbMV was very high and they were very widespread compared with FBNYV. The luteoviruses and PSbMV were the most common viruses of grass pea in this region. These viruses, together with BYMV, were found for the first time on grass pea in Ethiopia during this survey. Abraham and Lencho (2000) identified FBNYV in grass pea plants with severe stunting, although no virus was recovered from samples with yellowing symptoms. The occurrence of the luteoviruses, PSbMV and BYMV, in addition to FBNYV, in the present study may be associated with yellowing, chlorosis and mosaic symptoms. In the present study, the large sample size, as well as the more rigorous sampling method used (involving the collection of both random and symptomatic samples), was likely to have produced more realistic results than the previous study (Abraham and Lencho, 2000), which used small sample sizes and considered only symptomatic samples.

In the present study visual scoring underestimated the true incidence of infection. This finding is consistent with Tadesse *et al.* (1999) and Abraham *et al.* (2000). To gain a better understanding of the incidence and importance of viruses in a field, field scoring for virus infection should be supplemented with laboratory testing of random samples.

In this study, twenty-six (7 chickpea and 19 lentil) samples were positive to BBMV (Table 3). Both chickpea and lentil are known to be susceptible to this beetle-transmitted virus (Fortass and Diallo, 1993; Makkouk and Kumari, 1995), and BBMV has been reported to occur naturally on food legumes in many countries in West Asia and

North Africa (Makkouk *et al.*, 1988; Fortass and Bos, 1992). However, this is the first report of BBMV affecting legumes in Ethiopia.

SbDV, BYMV, PSbMV and BWYV have previously been reported in Ethiopia (Tadesse *et al.*, 1999; Abraham *et al.*, 2000), but this is the first report, in Ethiopia, of the natural infection of faba bean, pea and fenugreek with SbDV; of fenugreek with BWYV; and of grass pea with BYMV, PSbMV and BWYV. It is also the first report of BBMV infecting legume crops in Ethiopia.

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## Literature cited

- Aapola A.A. and G.I. Mink, 1973. Potential aphid vectors of pea seed-borne mosaic virus in Washington. *Plant Disease Reporter* 57, 552.
- Abraham A. and A. Lencho, 2000. Grass pea severe stunt virus disease in Ethiopia. *Pest Management Journal of Ethiopia* 4(1&2), 89–95.
- Abraham A. and K.M. Makkouk, 2002. The incidence and distribution of seed-transmitted viruses in pea and lentil seed lots in Ethiopia. *Journal of Seed Science and Technology* 30, 567–574.
- Abraham A., K.M. Makkouk, D. Gorfū, A.G. Lencho, K. Ali, N. Tadesse, A. Yusuf and A. Lencho, 2000. Survey of faba bean (*Vicia faba*) virus diseases in Ethiopia. *Phytopathologia Mediterranea* 39, 277–282.
- Amare G. and S.P.S. Beniwal, 1988. Faba bean and field pea research in Ethiopia: Development, achievements and future strategies. In: *Proceedings of the 19th National Crop Improvement Conference*, 22–26 April 1987, IAR, Addis Ababa, Ethiopia, 189–228.

- Bos L., R.O. Hampton and K.M. Makkouk, 1988. Viruses and virus diseases of pea, lentil, faba bean and chickpea. In: *World Crops: Cool Season Food Legumes* (R.J. Summerfield, ed.), Kluwer Academic Publishers, Dordrecht, the Netherlands, 591–615.
- Central Statistical Authority (CSA) of Ethiopia, 2002. Central Agricultural Census Commission, Ethiopian Agricultural Sample Enumeration, 2001–2002. In: *Report on the Preliminary results of area, production and yield of temporary crops (private peasant holdings, Meher season)*, Part I, October 2000, Addis Ababa, Ethiopia, 38–40.
- Central Statistical Authority (CSA) of Ethiopia, 2004. Report on the area, production and yield of crops, agricultural sample survey, 2003–2004, Meher season. CSA, Volume I, Statistical Bulletin 302, May 2004, Addis Ababa, Ethiopia.
- Cockbain A.J., 1983. Viruses and virus-like disease of *Vicia faba* L. In: *The Faba Bean (Vicia faba L.) a Basis for Improvement* (P.D. Hebbethwaite, ed.). Butterworths, London, UK, 421–462.
- Fortass M. and L. Bos, 1992. Broad bean mottle virus in Morocco; variability, interaction with food legume species, and seed transmission in faba bean, pea, and chickpea. *Netherlands Journal of Plant Pathology* 98, 329–342.
- Fortass M. and S. Diallo, 1993. Broad bean mottle virus in Morocco; curculionid vectors, and natural occurrence in food legumes other than faba bean (*Vicia faba*). *Netherlands Journal of Plant Pathology* 99, 219–226.
- Franz A., K.M. Makkouk, L. Katul and H.J. Vetten, 1996. Monoclonal antibodies for the detection and differentiation of faba bean necrotic yellows virus isolates. *Annals of Applied Biology* 128, 255–268.
- Gonzalez L.C. and D.J. Hagedorn, 1971. The transmission of pea seed-borne mosaic virus by three aphid species. *Phytopathology* 61, 825–828.
- Habtu A. and D. Gorfufu, 1986. Review of pulse disease research in Ethiopia. In: *A Review of Crop Protection Research in Ethiopia* (A. Tsedeke, ed.). *Proceedings of the First Ethiopian Crop Protection Symposium*, 4–7 February 1985, Addis Ababa, IAR, Ethiopia, 347–401.
- Hampton R.O. and F.J. Muehlbauer, 1977. Seed transmission of seed-borne virus in lentils. *Plant Disease Reporter* 61, 235–238.
- Katul L., 1992. *Characterization by Serology and Molecular Biology of Bean Leaf Roll Virus and Faba Bean Necrotic Yellows Virus*. Ph.D. thesis, University of Göttingen, Germany, 115 pp.
- Kumari S.G. and K.M. Makkouk, 1995. Variability among twenty lentil genotypes in seed transmission rates and yield loss induced by pea seed-borne mosaic potyvirus infection. *Phytopathologia Mediterranea* 34, 129–132.
- Makkouk K.M. and A. Comeau, 1994. Evaluation of various methods for the detection of barley yellow dwarf virus by the tissue-blot immunoassay and its use for virus detection in cereals inoculated at different growth stages. *European Journal of Plant Pathology* 100, 71–80.
- Makkouk K.M. and S.G. Kumari, 1995. Transmission of broad bean stain comovirus and broad bean mottle bromovirus by weevils in Syria. *Journal of Plant Disease and Protection* 102(2), 36–139.
- Makkouk K.M. and S.G. Kumari, 1996. Detection of ten viruses by the tissue-blot immunoassay (TBIA). *Arab Journal of Plant Protection* 14(1), 3–9.
- Makkouk K.M., L. Bos, A. Rizkallah, O.I. Azzam and L. Katul, 1988. Broad bean mottle virus: Identification, serology, host range and occurrence on faba bean (*Vicia faba*) in West Asia and North Africa. *Netherlands Journal of Plant Pathology* 94, 195–212.
- Makkouk K.M., S.G. Kumari and L. Bos, 1993. Pea seed-borne mosaic virus: Occurrence in faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Med.) in West Asia and North Africa, and further information on host range, transmission characteristics, and purification. *Netherlands Journal of Plant Pathology* 99, 115–124.
- Makkouk K.M., S.G. Kumari, J.d'A. Hughes, V. Muniyappa and N.K. Kulkarni, 2003. Other legumes: Faba bean, chickpea, lentil, pigeonpea, mungbean, blackgram, lima bean, horegram, bambara groundnut and winged bean. In: *Virus and Virus-like Diseases of Major Crops in Developing Countries* (G. Loebenstein, G. Thottappilly, ed.), Kluwer Academic Publishers, Dordrecht, the Netherlands, 447–476.
- Tadesse N., K. Ali, D. Gorfufu, A. Abraham, A. Lencho, M. Ayalew, A. Yusuf, K.M. Makkouk and S.G. Kumari, 1999. Survey for chickpea and lentil virus diseases in Ethiopia. *Phytopathologia Mediterranea* 38, 149–158.

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