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Survey of faba bean (*Vicia faba* L.) virus diseases in Ethiopia

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Summary. Surveys conducted in 1996 and 1997 to assess the status of virus diseases affecting faba bean in the major growing areas of Ethiopia indicated that leaf yellowing, rolling, necrosis and stunting were the most common disease symptoms. The highest visually-observed disease incidence in a field was 85%, recorded in the Wello region (north-eastern Ethiopia). When 3049 symptomatic samples collected from 211 fields from all over Ethiopia were tested by the tissue blot immunoassay (TBIA) for 14 viruses, 1592 samples (52.2%) were found to be infected with at least one virus. Faba bean necrotic yellows virus (FBNYV, genus *Nanovirus*) was the most frequent (63.2%), followed by luteoviruses (28.5%) [such as Beet western yellows virus (BWYV, genus *Polerovirus*, family *Luteoviridae*) and Bean leaf roll virus (BLRV, family *Luteoviridae*)] and Chickpea chlorotic dwarf virus (CCDV, genus *Mastrevirus*, family *Geminiviridae*) (3.1%). Mosaic/mottling symptoms were observed in some fields but incidence was always very low (<1%). The mosaic/mottling causing viruses identified were Broad bean stain virus (BBSV) and Broad bean true mosaic virus (BBTMV) (genus *Comovirus*, family *Comoviridae*), Pea seed-borne mosaic virus (PSbMV) and Bean yellow mosaic virus (BYMV) (genus *Potyvirus*, family *Potyviridae*). Testing of 3035 random samples collected in 1997 from 37 fields in the Shewa and Arsi regions revealed 1.6% luteovirus and 0.6% FBNYV infection, indicating that the incidence of these viruses on faba bean is very low in central Ethiopia. The survey suggested that virus diseases are currently of economic importance on faba bean in north-eastern Ethiopia and potentially important in the other areas, with FBNYV and luteoviruses being the most important.

Key words: Ethiopia, faba bean, legumes, *Vicia faba,* virus diseases.

Introduction

Faba bean (*Vicia faba* L.) is the most widely grown food legume in Ethiopia with an estimated annual production of 285,000 metric tonnes obtained from a cultivated area of some 245,000 ha. Worldwide, Ethiopia is the second largest producer in area and production after China (FAO, 1996). However, yield in the country is generally very low, and diseases represent one of the major production constraints (Gorfu and Beshir, 1994).

Several viruses are known to infect faba bean worldwide, with some causing serious economic losses (Bos *et al*., 1988; Makkouk *et al*., 1988; Katul *et al*., 1993). In Ethiopia, a great variety of virus-like symptoms have been observed on faba bean, but only a few viruses have been identified. BLRV was reported on the basis of symptoms observed

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(Bos, 1974). Some viruses were identified from faba bean in studies based, however, on a small number of samples and collected from few locations. These include PSbMV (Makkouk *et al*., 1993; Abraham and Albrechtsen, 1998) and FBNYV (Franz *et al*., 1997). Accordingly, the existing information on viruses and virus diseases of faba bean in Ethiopia is rather limited, and no meaningful conclusions can yet be drawn about their significance and economic impact on production. This paper presents the results of a 2-year survey conducted in 1996 and 1997 on the occurrence, importance, and distribution of viruses and virus diseases of faba bean in the major growing areas of Ethiopia.

Materials and methods

Field inspection and sample collection

Field surveys were conducted in the major faba bean growing regions of Ethiopia, Shewa, Arsi, Bale, Wello, Gojam, Gonder and Tigray in 1996 and 1997. Fields inspected were selected on the basis of predetermined distances between fields, on average 5-10 km apart (Fig. 1). For sample collection and determination of disease incidence in the fields, two methods were used. During the 1996 survey which included all the provinces (Survey I), virus incidence was determined in each field on the basis of visual symptoms by counting the number of symptomatic plants in a sample of 100 plants selected from random locations in the field. In addition, 10-20 symptomatic plants were collected for virus testing in the laboratory. Another survey (Survey II) was carried out in 1997 in the Shewa and Arsi regions. To determine virus incidence in the field in this latter survey, two methods were employed: (i) visual estimation as described above and (ii) 50-120 random samples were collected from each field and tested in the laboratory for the presence of viruses. Symptomatic plants were also collected from the field and were classified for convenience into two categories: (i) those with leaf rolling/yellowing/stunting/necrosis and (ii) those with mosaic/mottle. The number of symptomatic samples collected in each category depended on its relative abundance in the field, from none to 20 samples per field.

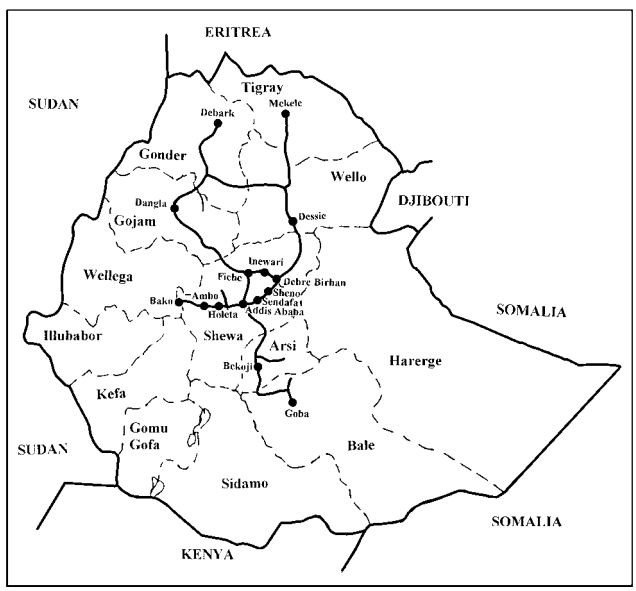

Fig. 1. A map of Ethiopia showing the location of the regions surveyed for faba bean virus diseases. Dotted lines indicate regional boundaries whereas solid lines indicate survey routes.

Serological tests

All the symptomatic (from Surveys I and II) and randomly collected (Survey II) samples were serologically tested by tissue blot immunoassay (TBIA) for presence of the 14 viruses known to infect faba bean. For this assay, rabbit polyclonal antisera to Alfalfa mosaic virus (AMV, genus *Alfamovirus*, family *Bromoviridae*), Cucumber mosaic virus (CMV, genus *Cucumovirus*, family *Bromoviridae*), BBSV, BYMV, BBTMV, Broad bean wilt virus (BBWV, genus *Fabavirus*, family *Comoviridae*), Broad bean mottle virus (BBMV, genus *Bromovirus*, family *Bromoviridae*), PSbMV, Pea enation mosaic virus (PEMV, genus *Enamovirus*, family *Luteoviridae*), Pea early browning virus (PEBV, genus *Tobravirus*), BWYV, BLRV and CCDV were used. In addition, two monoclonal antibodies were used: one for the detection of FBNYV and the other (5G4), is a broad spectrum monoclonal antibody which react with all legume luteoviruses (members of the family *Luteoviridae*). Antisera were provided by ICARDA, Virology Laboratory, Syria. Goat anti-rabbit- and goat antimouse-alkaline phosphatase conjugates and enzyme substrates were obtained from Sigma Chemical Company. The TBIA was conducted as described by Makkouk and Comeau (1994) with one modification, in that the nitrocellulose membrane was pretreated with 2% Na_2SO_3.

Results and discussion

Yellowing, rolling, stunting and necrosis were the most frequently observed symptoms in most faba bean growing areas. Table 1 shows for each area the number of fields with virus occurrence and the highest incidence recorded in that area (Survey I). Virus incidence based on visual estimation ranged from 0-85%. However, there was a wide variation in virus occurrence and incidence in different areas across the country. In north-eastern Ethiopia, particularly south and north Wello, virus diseases were prevalent in 18 out of 23 (78.3%) of all fields having visible infections, and reached 85% in one of these fields. On the other hand, although viruses were detected in more than half of the fields inspected in the regions surveyed, incidence was low (0- 20%) in most of them. Exceptions included some fields in restricted areas in north Shewa (Ankober), south Gonder (Gonder Zuria), and Arsi (Asasa) (Fig. 1) where a maximum incidence of 25-85% was recorded (Table 1).

Laboratory testing showed that at least one virus was detected in 86 out of the 176 fields tested, whereas in the remaining 90 fields no virus was detected.

In this study, out of 3049 symptomatic samples collected from 211 fields (176 in Survey I and 35 in Survey II), 1592 samples (52.2%) were positive for at least one virus (Tables 2 and 3). Among the infected samples, FBNYV was the most frequent virus (63.2%), followed by the luteoviruses (28.5%), an indication that these viruses are the most important on faba bean in Ethiopia.

There was also variation in the incidence of FBNYV and the luteoviruses in different geographical areas. FBNYV seemed to be more frequent in north-eastern and south-eastern Ethiopia, while the luteoviruses (Tables 2 and 3) were more common in central Ethiopia, particularly in the Shewa region. The yellowing, rolling and stunting symptoms observed at a high rate in the Wello and Ankober region of north Shewa and in the Asasa region of Arsi (Fig.1) were confirmed by laboratory testing to be exclusively caused by FBNYV.

Some of the samples that were positive with the broad-spectrum legume luteovirus monoclonal antiserum (5G4) were tested with polyclonal antibody against BWYV and BLRV and were found positive to one, or both. However, since the BWYV polyclonal antiserum used is known to cross-react with a large number of luteoviruses, the exact identity of these viruses needs to be confirmed using specific monoclonals. Accordingly, in this study, the luteoviruses are reported as a group. CCDV was also detected in some (3%) of the samples with yellowing, rolling, stunting and necrosis symptoms. Mosaic/mottle symptoms, although observed in many fields, were found to be present at only a very low rate (<1%). In such samples, BYMV, PSbMV, BBSV and BBTMV were identified. From all virus-infect-

Table 1. Incidence of virus infections in faba bean in different production areas in Ethiopia during 1996.

Area	No. of districts covered	No. of fields surveyed	Virus infection	
			No. of fields affected	Highest incidence recorded (%)
Arsi	5	28	4	25
Bale	4	12	11	10
North Shewa	4	32	11	85
West Shewa	5	19	1	10
East Gojam	3	8	4	2
West Gojam	3	10	10	10
South Wello	3	13	10	80
North Wello	5	10	8	85
South Gonder	3	10	6	25
North Gonder	5	17	7	40
South Tigray	4	17	14	5
Total	44	176	86	-

Table 2. Results of serological tests of symptomatic samples collected from 176 faba bean fields in the major production areas of Ethiopia during 1996.

Area	No. of samples collected	No. of samples infected with[a]						
		FBNYV	Luteoviruses	CCDV	PSbMV	BBSV	BYMV	BBTMV
Arsi	440	152	54	6	0	5	2	0
Bale	125	70	12	0	4	0	5	1
North Shewa	306	55	95	4	0	3	4	0
West Shewa	255	42	73	8	2	0	0	1
East Gojam	30	0	22	0	0	0	0	2
West Gojam	152	31	0	0	0	1	2	0
South Wello	284	145	36	0	4	0	0	3
North Wello	340	205	42	1	1	0	2	0
South Gonder	176	102	25	2	0	0	1	0
North Gonder	220	73	30	0	0	1	0	0
South Tigray	235	95	23	6	1	0	0	0
Total	2563	970	412	27	12	10	16	9

[a] All samples were negative to AMV, BBMV, BBWV, PEBV, PEMV, CMV.

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

ed samples, only 3.1% were shown to host one of these viruses. No virus was identified in nearly half (47.8%) of the symptomatic samples tested, suggesting either that some viruses were undetected by the antisera used or that the symptoms were caused by factors other than viruses.

When virus incidence as determined by visual symptoms was compared with that obtained by laboratory (serological) testing of random samples in the 37 fields surveyed in 1997, a virus incidence of 5% or less was found in 84% of fields inspected for visual symptoms and 81% of fields serologically tested (Table 4). In a few cases, however, laboratory testing indicated that visual inspection underestimated the infection level in a field. Two fields that were found to have infection levels higher than 21% in the laboratory were placed in a lower incidence category by visual inspection. This suggested that visual scoring as done for most of the fields surveyed in this paper could sometimes underestimate the actual incidence. It is therefore important to supplement visual scoring with laboratory testing of random samples to have a better understanding of virus incidence in a field.

FBNYV, a recently described virus from West Asia and North Africa including Ethiopia, is persistently transmitted by aphids and has a wide host range within the Leguminosae (Katul *et al*., 1993; Franz *et al*., 1996). In Egypt, Jordan, Lebanon, and Syria, FBNYV has been known to cause the main viral disease of faba bean. During 1992, a serious virus epidemic caused by faba bean necrotic yellows virus affected faba bean crops in middle Egypt, with yield losses of over 90% (Makkouk *et al*., 1994). Almost the same epidemic occurred in 1999 (K. Makkouk, unpublished). The present report shows that FBNYV is a widespread and economically important virus in some parts of Ethiopia and is potentially important in others. Since FBNYV has also been detected in Ethiopia in both lentil and chickpea (Tadesse *et al*., 1999), it poses a potential threat for the production of major legume crops in the country.

BLRV and BWYV cause fairly similar symptoms on legumes, but the latter has a wider host range and more aphid vectors (Brunt *et al*., 1996). Lute-

Table 3. Results of serological tests on samples collected from 37 fields in the Shewa and Arsi provinces in 1997.

Region	Sample collection method	No. of fields surveyed	No. of samples collected	No. of samples found positive to [a]						Average incidence (%)[b]
				FBNYV	Luteo-virus	CCDV	PSbMV	BBSV	BYMV	
Ambo-Holetta	Symptoms	8	100	6	9	7	0	1	0	
	Random	8	615	4	22	12	0	0	0	6.2
Addis-Bekoji	Symptoms	8	110	20	30	10	0	0	0	
	Random	8	620	15	22	9	0	0	0	7.4
Addis-Sendafa	Symptoms	3	45	10	0	0	3	0	0	
	Random	4	230	0	0	0	1	0	0	0.4
Sendafa-Sheno	Symptoms	1	15	0	1	1	0	0	0	
	Random	1	120	0	0	0	0	0	1	0.8
Sheno-Debre	Symptoms	2	30	0	1	1	0	0	0	
Berhan	Random	2	180	0	0	2	0	0	0	1.1
Debre Berhan-	Symptoms	6	89	1	1	1	0	0	0	
Inewari	Random	7	670	0	0	0	0	0	0	0
Addis-Fiche	Symptoms	7	97	0	0	1	0	0	0	
	Random	7	600	0	5	0	0	1	0	1.0
Total	Symptoms	35	486	37	42	21	3	1	0	
	Random	37	3035	19	49	23	1	1	1	3.1

[a] All samples were negative to AMV, BBMV, BBWV, PEBV, PEMV, CMV (see Table 2).
[b] Total incidence was calculated only from samples collected at random.

Table 4. Comparison between virus incidence in 37 faba bean fields as determined by visual inspection in the field and as determined by laboratory (serological) testing of randomly collected samples from the Shewa and Arsi regions, in 1997.

Type of identification	No. of fields in disease incidence (%) category			
	< 1	1-5	6-20	21-50
Field symptoms	8	23	6	0
Laboratory testing	26	4	5	2

oviruses have been reported as being economically important viruses of faba bean in many European countries, New Zealand, Middle East and North Africa, including Sudan (Bos *et al*., 1988). This paper confirms their importance on faba bean in Ethiopia. The effect of individual luteoviruses on faba bean production in this country should be studied further.

CCDV, detected in a few samples in this study, is a newly described Mastrevirus from India. It is transmitted by a leafhopper (Cicadellidae: *Orosius orientalis*) from chickpea plants showing symp-

toms of stunting and yellowing, previously attributed to BLRV (Horn *et al*., 1993). More recently, Makkouk *et al*. (1995) reported the natural occurrence of CCDV in faba bean and chickpea in Sudan. The present study extends this information by reporting it also on faba bean in Ethiopia.

The occurrence of FBNYV and luteoviruses in Ethiopia and their high incidence in some locations, such as Wello, is probably due to the presence of effective vectors and environmental conditions favouring their population buildup and movement. Depending on the environmental conditions and the availability of alternate hosts, the deleterious effect of viruses can vary from season to season in the same area. Detailed surveys to monitor changes in virus incidence from one year to another should be continued for 3-4 years.

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