Seed-borne Viruses Infecting Some Important Crops in Ethiopia

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Abstract

Seed samples comprising haricot bean, cowpea, faba bean, field pea, lentil, peanut, hot pepper, lettuce, and leaf samples of maize collected from various locations in Ethiopia were tested for a total of 16 seed-borne viruses. Visual inspection, growing-on test, ELISA, indicator plant methods and electron microscopy were employed. The viruses detected were bean common mosaic virus in haricot bean, soybean mosaic virus in soybean, pea seed-borne mosaic virus in faba bean and field pea, tobacco mosaic virus in hot pepper, lettuce mosaic virus in lettuce, and sugar cane mosaic virus in maize. The presence of pea seed-borne mosaic virus in field pea and lettuce mosaic virus in lettuce are confirmed for the first time in Ethiopia. No virus was detected in lentil, cowpea and peanut seed samples tested.

Introduction

Seed transmission is one of the important methods for plant virus dissemination and survival in nature and thus plays an important role in the ecology of many crop virus diseases. Some viruses such as barley stripe mosaic virus are transmitted naturally only by seeds while the majority of seed-borne viruses are transmitted both by seeds and vectors, including arthropods, fungi and nematodes.

Seed-borne viruses are of great economic importance; first, because plants developed from infected seeds may suffer from infection that results in direct quantitative or qualitative yield loss. Second, the infected seeds may act as a primary source of infection in a field from which viruses are spread by insect vectors to plants in the same or nearby fields. In addition, seeds may act as a means for the spread of viruses or their strains from one area to another where they are not present before through international exchange of germplasm or seed trade (Neergaard 1979). Hence, the detection and identification of seed-borne viruses in a country is a prerequisite for their control which can be achieved by producing virus-free seeds in selected areas or by establishing seed certification schemes, and by setting up quarantine lists.

In Ethiopia, there is some information on seed-borne diseases caused by fungi (Awgechew 1992) but little is known about viruses associated with crop seeds. In this paper, seed-borne viruses detected and identified from some crop seeds grown in Ethiopia are reported.

Materials and Methods

Fifty-four seed samples comprising haricot bean (*Phaseolus vulgaris*), faba bean (*Vicia faba*), soybean (*Glycine max*), field pea (*Pisum sativum*), lentil (*Lens culinaris*), cowpea (*Vigna unguiculata*), and hot pepper (*Capsicum annuum*) were collected during the 1993 crop season from various locations in Ethiopia.

Discussion

The data show that the leafminer *Sphaeroderma guizotiae* is a serious pest of noug that can cause heavy leaf destruction on noug. The pest problem is rather important in outbreak seasons like the one in 1995 where up to 35% leaflet damage (burning of the entire leaves) and 45% leaf area damage were recorded. The planting of the crop early (about the beginning of June) at Adet has proveN to be of advantage to reduce the pest attack and at the same time obtain increased seed yield.

Control of pests by manipulating sowing date has several advantages. It is a simple cultural practice which is affordable and safe, and also serves as one component for the future development of an integrated management of the pest. The decreasing trend of the pest after the end of August indicates, among other factors, that may be associated with an increase in the activity of internal parasitoids. Other environmental factors such as temperature or rainfall might also be responsible. Besides, the growth stage of the crop may also be another possible factor associated with the reduction in the pest damage towards the end of the season. These are subject to further study. This sowing date adjustment, supplemented with the future studies on the role of leafminer natural enemies. will lead to an integrated pest management approach that makes use of naturally existing, environmentally friendly control options.

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from the Adet area. The IIE report also indicated that this species was originally described from Addis Ababa, near the Little Akaki River (2300 m above sea level) apparently mining leaves of *Guizotia schimperi*, a relative species of noug. It has also been observed mining leaves of the weed *Guizotia scabra* in and around Adet (Melaku & Amare 1996). According to the IIE report this pest is known only from Ethiopia.

Our observations on farmers' fields suggested that early planted crop always seemed to be less vulnerable to the attack by this pest and this simple observation stimulated us to undertake this study. Adjusting sowing date is a simple choice for resource-poor farmers where no additional investment is needed.

Materials and Methods

The experiment was carried out during the 1994 and 1995 cropping seasons at Adet Research Center experimental field on black soil. It was laid out in a randomized complete block design replicated three times. The plot size was 5 m x 1.8 m, consisting of 6 rows spaced 30 cm apart, with 1 m spacing between plots. The improved noug variety 'Fogera-1' was drilled at the recommended seeding rate of 10 kg ha^{-1}. The treatments consisted of five sowing dates at 10-day intervals, beginning on May 30 and ending on July 14. Sampling began at about two months after sowing and continued every week (in 1994) or every two weeks (in 1995). Five plants per plot were randomly selected and the number of leaflets damaged and undamaged were counted, recorded, and percentage leaf damage was thus calculated.

Estimated leaf area damage was also recorded on a sample of 10 leaflets per plot in 1995 once at the peak stage of infestation.Grain yield data were taken from the central four rows. The data thus recorded were subjected to analysis of variance.

Results

The leaflet damage significantly varied from season to season. It was lower in 1994 than in 1995. Maximum leaflet damage in 1994 and 1995 was 11 and 35 percent, respectively (Tables 1 & 2). Infestation increased with the delay in planting while at the same time the yields significantly decreased (Table 3).

As sampling was continued for each sowing date separately, infestations generally decreased after August 23. This showed that the highest infestation is expected at about the last week of August. It steadily decreased thereafter as can be seen on Tables 1 and 2.

Leaf area damage ranged between 23 and 45 percent (Table 4). The maximum record of leaf area damage was recorded from the last sowing date of July 14.

Table 1. Percentage leaflet damage caused by the leafminer *Sphaeroderma guizotiae* on noug at Adet, 1994.

Sowing date	Sampling date				
	9 Aug	24 Aug	31 Aug	7 Sept	14 Sept
May 30	0.6	0.5	1.5	0.0	3.2
June 10	2.5	0.0	2.1	0.4	0.8
June 20	2.5	5.1	4.5	1.9	4.9
June 30	0.0	11.2	10.0	7.9	8.8
July 10	0.0	2.4	0.0	0.5	2.5

Table 2. Percentage leaflet damage caused by the leafminer *Sphaeroderma guizotiae* on noug at Adet, 1995.

Sowing date	Sampling date		
	23 August	5 September	21 September
May 30	4.2	2.5	1.9
June 10	7.7	4.5	2.6
June 20	11.3	8.9	5.2
July 4	20.5	15.4	17.7
July 14	13.4	35.7	24.3

Table 3. Mean percentage leaflet damage caused by the leafminer *Sphaeroderma guizotiae* on noug and grain yield as influenced by sowing date at Adet, 1994 and 1995.

Sowing date 1994 (1995)	Leaflet damage		Yield (q ha-^{1})	
	1994	1995	1994	1995
May 30 (30)	1.2	2.9	6.5a	10.1a
June 10 (10)	1.2	4.9	6.7a	8.9ab
June 20 (20)	3.8	8.5	5.4ab	7.6b
June 30 (July 4)	7.6	17.9	5.2ab	7.6b
July 10 (July 14)	7.1	24.5	3.7b	7.4b
Mean			5.7	8.32
SE (+)			0.67	0.41
CV (%)			26.1	10.9

Means followed by the same letter are not statistically different from each other at the 5% probability level (Duncan's new multiple range test).

Table 4. Estimated percentage leaf area damage on noug caused by the leafminer *Sphaeroderma guizotiae* at Adet, 1995.

Sowing date	Damage
May 30	22.9b
June 10	33.0ab
June 20	27.5b
July 4	43.5a
July 14	46.3a

These, together with a previously collected lettuce (*Lactuca sativa*) seed sample, and nine maize leaf samples collected from western Ethiopia were tested for a total of 16 seed-borne viruses (Table 1) at the Danish Government Institute of Seed Pathology for Developing Countries, Denmark. The samples were tested for seed-borne viruses known to be of worldwide importance in the respective crops. Each seed sample was first visually inspected for symptoms suggestive of virus infection such as mottling, necrosis, or wrinkling. From each sample 360 seeds were sown in pots in a vector-proof greenhouse and the germinated seedlings were examined for virus-like symptoms. All samples were subjected to ELISA test. Leaves of 160 seedlings with virus-like symptoms or randomly selected healthy looking plants were sampled for the ELISA test for selected viruses. Leaf samples of maize were also tested by ELISA for sugar cane mosaic virus. For hot pepper, 50 seeds from each sample were rubbed with the buffer in a polythene bag as tobacco mosaic virus can not be obtained from seedlings by growing-on test.

Table 1. Crops and the virus(es) for which they were tested#

Crop	Sample size	Virus(es)	Source of antisera
Haricot bean (DGISP)	14	1) BCMV 2) CMV*	1) R.O. Hampton, USA 2) Danish Government Institute of Seed Pathology
Cowpea	4	1)CABMV 2)CMV 3)CPMV 4)SBMV	1) R.K. Bock, Kenya 2*,3) DGISP 4 ATCC (American type culture collection, USA)
Faba bean	5	1)BBSV 2)BBTMV 3)BYMV 4)PSbMV	1,2) DGISP, 3) ATCC, 4) PPRC (Plant Protection Research Center, Denmark)
Field pea	9	1) PSbMV, 2) BBSV	1) PPRC, Denmark 2) DGISP (Denmark)
Lentil	5	1) PSbMV, 2) BBSV	1) PPRC, Denmark 2) DGISP (Denmark)
Peanut	1	1) PStV, PMV	1,2) D.V.R. Reddy, India
Soybean	12	1) SMV, 2) SSV** (CMV strain)	1,2) ATCC, USA
Pepper	4	TMV	DGISP, Denmark
Lettuce		1	LMVDGISP, Denmark
Maize	6	SCMV	J. Vetten, Germany

#*Acronyms of virus names:*
BBSV = broad bean stain virus
BCMV = bean common mosaic virus
SMV = soybean mosaic virus
BBTMV= broad bean true mosaic virus
PSbMV = pea seed-borne mosaic virus
CPMV = cowpea mosaic virus
TMV = tobacco mosaic virus
SCMV = sugar cane mosaic virus
PeMoV = peanut mottle virus
BYMV = bean yellow mosaic virus
CMV = cucumber mosaic virus
SbSV = soybean stunt virus
CABMV = cowpea aphid-borne mosaic virus
PStV = peanut stripe virus
LMV = lettuce mosaic virus
SBMV = southern bean mosaic virus
* *The antiserum reacts weakly with soybean stunt strain but strongly with the other CMV strains*
** *The antiserum reacts strongly with soybean stunt strain but weakly with other CMV strains*

A method similar to the direct antigen coating indirect ELISA (Hobbs *et al.* 1987) using either of alkaline phosphatase or penicillinase conjugate was used for all the samples. The leaves were homogenized in a 0.05 M phosphate buffer with 0.02% sodium azide. Each sample homogenate (100 ml) was coated in two wells of a microtiter plate (Nunc, Maxisorp™, Denmark). After subsequent addition of specific antibody, enzyme conjugates, washing and incubation steps, the substrate was added and the colour change was monitored by ELISA reader (Titertek Multiskan Plus) at 405 nm for alkaline phosphate and at 620 nm for penicillinase conjugate, using bromothymol blue as reaction indicator for the latter (Sudarshana & Reddy 1989).

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Samples which showed virus symptoms by growing-on test and/or positive by ELISA for the respective viruses were confirmed either by examination under the JOEL JEM-100SX electron microscope or biologically by inoculation to selected diagnostic indicator plants: *Nicotiana glutinosa* for tobacco mosaic virus and *Chenopodium quinoa* for lettuce mosaic virus.

Results and Discussion

Six seed-borne viruses were detected in 16 of the seed samples and five maize leaf samples. No virus was detected in the other seed samples tested (see Table 4).

Visual inspection of seed samples showed that most of soybean seeds showed mottling suggestive of the mother plant infection with soybean mosaic virus (SMV). Two distinct types of mottling, *viz*. brown and black, were observed, depending on the crop variety. The proportion of mottled seeds varied among varieties, ranging from trace amounts to more than 50% (Table 2). A purple spot symptom induced by the infection of the mother plant by *Cercospora kikuchii* was also observed in many seed samples but could easily be distinguished from mottling due to SMV. Virus-like symptoms were not observed by visual inspection in the other seed samples of other crops tested.

On the growing-on test, all of the soybean samples with mottled seeds showed mosaic and downward folding of leaves in the first trifoliate leaves, typical of SMV infection. In two haricot bean seed samples, typical mosaic and leaf distortion due to bean common mosaic virus (BCMV) was observed in the first two leaves. Faba bean seedlings from one sample showed downward folding of leaves, typical of pea seed-borne mosaic virus (PSbMV). Seedlings from the lettuce seed sample showed a mosaic symptom typical of lettuce mosaic virus (LMV). Virus-like symptoms were not observed in seedlings from other seed samples.

Most of the samples which have shown positive reaction by ELISA (Table 3) were those where virus-like symptoms were observed by the growing-on test. Thus, BCMV, PSbMV, SMV and LMV were detected by ELISA in haricot bean, faba bean, soybean and lettuce, respectively. In addition, PSbMV was detected in one field pea seed sample although seedlings did not show clear virus-like symptom in the growing-on test. Direct examination of homogenate of one hot pepper seed sample by ELISA showed the presence of tobacco mosaic virus (TMV). Five maize leaf samples collected from the Uke, the Bako, the Anger Loko and the Lugo areas in western Ethiopia were shown to be positive to sugar cane mosaic virus (SCMV) by ELISA, using antiserum (Table 3). Examination of the leaf preparations under electron microscope for ELISA positive samples showed the presence of filamentous particles measuring about 750 nm in haricot bean, soybean, field pea and maize, confirming the presence of BCMV, SMV, PSbMV and SCMV respectively (Fig. 1-3). For LMV in lettuce, the ELISA result was confirmed by inoculation to *Chenopodium quinoa* plant which showed chlorotic local lesion in inoculated leaves and systemic infection in new leaves. When pepper seed homogenate from the sample positive by ELISA to TMV was inoculated to *Nicotiana glutinosa,* local lesions were observed, confirming the presence of TMV in the hot pepper seed sample. No virus was detected in the other seed samples of the crops mentioned above as well as seed samples of lentil, cowpea, and peanut tested.

It is apparent from the results that seed-borne viruses of worldwide importance are present in Ethiopia in many of the crops tested (Table 4). The occurrence and importance of BCMV in haricot bean, SMV in soybean, TMV in hot pepper and PSbMV in faba bean in Ethiopia has been reported earlier (Agranovsky 1985a, 1985b, Makkouk *et al*. 1993) although their association with seeds was not assessed. Lettuce mosaic virus, which is known to be the most important virus disease of lettuce worldwide, has been suspected in Ethiopia on the basis of visual observations (Bos 1974). Its presence in lettuce in Ethiopia is confirmed by our present study.

The pea seed-borne mosaic virus is reported here for the first time in field pea in Ethiopia. The virus is known to be the major virus contaminating field pea germplasm in many parts of the world (Khetarpal & Maury 1987). Sugar cane mosaic virus has recently become an important problem in maize in western Ethiopia. It was earlier detected from a few samples of maize around Harar (Abdulnasir et al. 1991). The virus is transmitted by seeds (at low rate) and aphids and is an important problem in maize in Eastern Africa (Louie 1980).

Table 2. Proportion of mottled seeds in nine soybean genotypes obtained from seed collection of Awassa Agricultural Research Centre, Ethiopia.

Genotype	Acc. No.*	Proportion of mottled seeds**
PK. 7386	36033	low
Braxton	36030	low
Grawford	34988	very high
ISRA/44A/73	36032	low
Kwankyo	36026	high
SB.PNVT	36031	very high
TGX-47.5C	36029	medium
Williams	34983	high
Wiliams 82	36027	high

**Refers to the accession number of the seed samples at the Danish Government Institute of Seed Pathology for Developing Countries;* low = < 5%; medium = 5-25%; high = 25-50%; very high = > 50%.

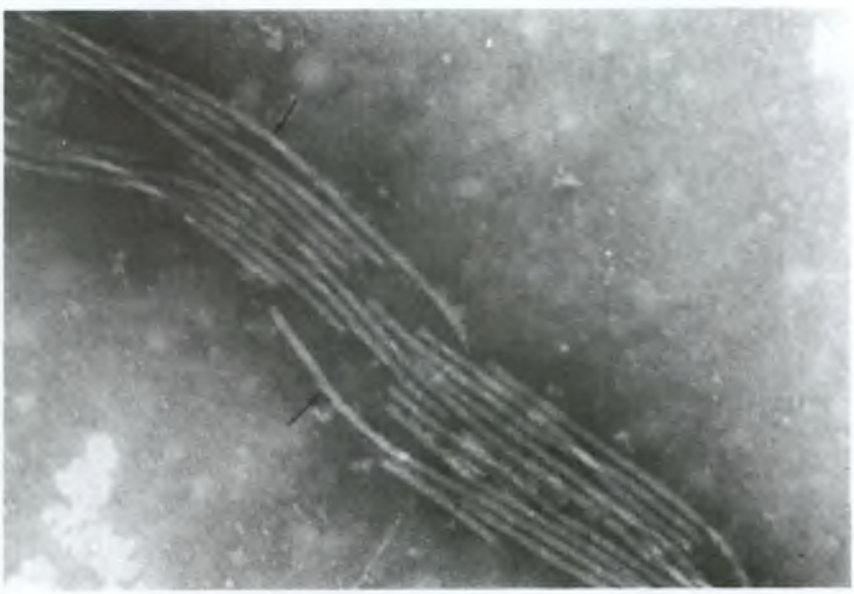

Fig. 1. Filamentous particles observed under electron microscope from pea leaves from seedlings infected with pea seed-borne mosaic virus(magnification x 120,000)

Fig. 2. Filamentous particles observed under electron microscope from soybean seedlings infected with soybean mosaic virus (magnification: x200,000).

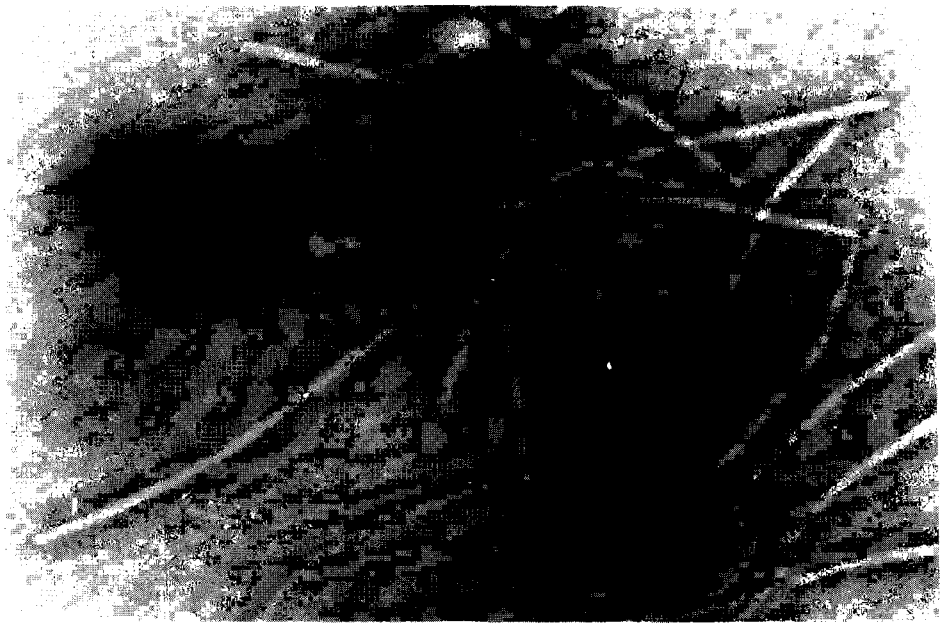

Fig. 3. Filamentous particles observed under electron microscope from bean leaves from seedlings infected by bean common mosaic virus (magnification x120,000)

Table 3. ELISA test results (optical readings) for selected virus/crop combination in which seed-borne viruses were detected by using alkaline phosphatase and/or penicillinase conjugates[Æ]

Virus/crop(Acc.#)	A_{405} (Alkaline phoshatase)			A_{620} (Penicillinase)		
	Healthy control	Threshold value	Test sample	Healthy control	Threshold value	Test sample
BCMV/bean(36044)	nt	nt	nt	1.28	0.43	0.13
PSbMV/ faba bean (36064)	0.08	0.24	0.98	nt	nt	nt
PSbMV/ field pea (36054)	0.03	0.09	0.65	nt	nt	nt
SMV/soybean(36026 & 36894)	0.04	0.12	0.84	1.34	0.45	0.09
TMV/pepper-(35285)	0.04	0.12	0.81	nt	nt	nt
LMV/lettuce(27404)	0.05	0.15	0.7	nt	nt	nt
SCMV/maize	0.07	0.21	1.15	nt	nt	

[Æ] *Numbers in the bracket refer to the accession number of the seed samples at the Danish Government Institute of Seed Pathology for Developing Countries;*
nt=not tested by that system;
threshold values were obtained by multiplying the mean absorbance of the healthy (negative) control by three (3Xnc) for alkaline phosphatase, and by dividing the mean of the healthy (negative) controls by three (Xnc/3) for penicillinase.

Table 4. Seed-borne viruses detected and confirmed in the crop samples from Ethiopia#.

Crop	Samples with virus			
	No. of samples with virus	Acc. No.	Place of collection	Virus detected
haricot bean	2	36040, 36044	Awassa	bean common mosaic virus
faba bean	1	36064	Ambo	pea seed-borne mosaic virus
field pea	1	36054	Ambo	pea seed-borne mosaic virus
soybean	10	Several	Ambo, Awassa	soybean mosaic virus
pepper	1	35285	Guder	tobacco mosaic virus
lettuce	1	27404	Kulumsa	lettuce mosaic virus
maize+	5	Several	Western Ethiopia	sugar cane mosaic virus

#=seed samples of cowpea, lentil, peanut and the remaining samples where no viruses were detected are not included; +=leaf (not seed) samples tested.

The fact that the major seed-borne viruses are detected in the crop seeds tested indicates the possibility that the viruses are widely distributed in the country, being disseminated by infected seeds. The absence of the viruses in cowpea, lentil and peanut seed samples tested in this study can only indicate that the samples tested are free of the viruses they are tested for. More intensive surveys in large number of seed samples are necessary to confirm the presence or absence (and importance) of these viruses.

Except for TMV, which is transmitted by contact during cultural operation, all the viruses detected in this study are also transmitted by aphids. In ecological sense, it means that the plants grown from infected seeds serve as primary source of infection which is further spread by the aphid vectors to other plants in the same or adjacent field.

In such cases, very few infected seeds can induce an epidemic and can result in total crop failure. For example, lettuce production in California was severely affected by lettuce mosaic virus when even trace incidence of infected seeds (0.001%) occurred because of subsequent spread by aphid vectors (Grogan 1983). Furthermore, the introduction of exotic germplasm into Ethiopia for use in crop improvement programmes is continually increasing with the attendant risk of introducing serious seed-borne viruses (or their strains) which are not present in the country. These viruses introduced at early stage of the breeding

programme will be multiplied and carried on to the later stages of seed production. Recognition of these problems and testing seeds for viruses during germplasm introduction, coupled with the use of virus-free seed, will facilitate the control of seed-borne viruses. In this regard, the start made by the national haricot bean programme recently to test haricot bean seeds imported to the country to avoid the introduction of the necrotic strains of BCMV, which are presumed to be absent in Ethiopia, is a good step and should be implemented for other crops known to be attacked by serious viruses.

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