Alfalfa mosaic virus: occurrence and variation among isolates from forage legumes in Ethiopia

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Abstract

Field samples and seedlings of 51 leguminous forage species were tested for alfalfa mosaic virus (AMV) in routine monitoring of seed multiplication fields of the International Livestock Research Institute (ILRI), Ethiopia. The virus was detected in 24 species and a solanaceous weed *Solanum nigrum*. Of these, the following 17 species are being reported for the first time as hosts of the virus in world literature: *Aeschynomene falcata, Centrosema pascuorum, Chamaecrista rotundifolia, Desmanthus virgatus, Desmodium intortum, Leucaena leucocephala, Macroptilium atropurpureum, Macroptilium lathyroides, Macrotyloma axillare, Medicago truncatula, Neonotonia wightii, Sesbania sesban, Stylosanthes scabra, Trifolium calocephala, T. steudneri, T. tembense* and *Vigna parkeri.* The solanaceous weed *S. nigrum* was suspected to play a role in the virus epidemiology. The level of seed infection varied from 0.00% to about 13.00%. Infectivity studies on 5 isolates of the virus revealed heterogeneity in prevalent AMV isolates. Two major strains were identified, based on the development of necrotic local lesions on *Vigna unguiculata* accessions. One of the isolates which failed to incite local necrotic lesions on *V. unguiculata* infected *Phaseolus vulgaris* cv. Top Crop systemically. Differences among isolates were also noticed in their concentration in different hosts as indicated by ELISA absorbance values.

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Introduction

Leguminous forages are an important source of protein for supplementation of low quality native grassland and crop residues for livestock production. They are also important for soil stabilisation and nitrogen fixation. The International Livestock Research Institute (ILRI) maintains over 13 000 accessions of grass and legume forages in trust in its genebank with the aim of making seeds freely available for evaluation and subsequent incorporation into sustainable crop-livestock systems (Hanson 1995). Distribution of seeds has been identified as a cause of long-distance spread of seed-borne viruses, especially in legumes (Hampton *et al.* 1982; Bos and Makkouk 1993). Among the seed-borne viruses infecting legumes, alfalfa mosaic virus (AMV) is one of the most important, occurring worldwide and recorded as naturally infecting over 150 herbaceous and woody species in 22 plant families (Jaspars and Bos 1980). Loss of herbage yield due to AMV amounts to 13–35% in *Medicago polymorpha* (Jones and Nicholas 1992), over 50% in *M. truncatula* (Dall *et al.* 1989) and 20–49% in *Trifolium subterraneum* (Jones 1992). The parallel losses in seed yield are 7–32%, 58–76% and 15–30%, respectively. The virus has also been implicated in reduced nitrogen fixation in nodules of *M. polymorpha* (Wroth *et al.* 1993). These effects usually depend on virus strain among other factors (Dall *et al.* 1989).

In Africa, AMV has been isolated from *Capsicum annuum* in Morocco (Lockhart and Fischer 1976), *Lablab niger* (syn. *L. purpureus*), *Vicia faba*, *Medicago sativa* and *Sonchus cornutus* in Sudan (Nour and Nour 1962), *Phaseolus vulgaris* in South Africa (Neveling 1956), *Medicago sativa* in Tanzania and *Solanum tuberosum* in Kenya (Kaiser and Robertson 1976).

The ILRI seed multiplication fields are routinely monitored for seed-borne diseases to

make sure that only seeds of satisfactory phytosanitary status are distributed to recipients in geographically diverse areas. This paper reports some forage legume hosts of AMV found during the monitoring process and the variability among 5 isolates of the virus from 3 of these species.

Materials and methods

Field survey and seed health testing

Leaf samples were collected from field plants showing virus-like symptoms. These were tested for AMV by the antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) by an earlier protocol (Mih *et al.* 1995) using polyclonal antibodies obtained commercially from Agdia Inc., USA. Samples were extracted (1:10 w/v) in 0.05M carbonate buffer pH 9.6, containing 2% (w/v) polyvinylpyrrolidone and 0.1% (w/v) Na_2SO_3. Blocking was for 45min at 27°C with freshly prepared 3% (w/v) skim milk in phosphate-buffered saline (0.137M NaCl, 1.5mM KH_2PO_4, 8mM Na_2HPO_4, 3mM KCl, 3mM NaN_3) containing 0.05% v/v Tween 20 (PBST). Samples were tested in duplicate wells along with 4 negative (healthy) and 2 positive (a lucerne isolate of AMV maintained on seedlings of *Nicotiana glutinosa*) controls. Virus-bound antibodies were detected with alkaline phosphatase conjugate of goat anti-rabbit IgG (Sigma Inc.) diluted 1/2000 in PBST. The ρ-nitrophenyl-phosphate substrate (1 mg/ml 10% diethanolamine, pH 9.8) was incubated for 60 min at 27^0C before plates were read at 405nm wavelength (A_{405}) with a BIO-RAD model 2550 EIA reader. Each plate was blanked with the substrate buffer. A sample was considered diseased if the A_{405} value was at least twice that of the mean of the healthy controls. Positive results were confirmed by visual inspection.

For seed health testing, samples of 200–500 seeds from the ILRI genebank (scarified where necessary) were sown in seed trays filled with forest top soil. Germinated seedlings were observed for virus symptoms until the first non-juvenile leaves were fully expanded. Composite samples each comprising 10 leaves (1 leaf/plant) were tested for AMV by ACP-ELISA. The level of seed infection was estimated by the method of Gibbs and Gower (1960) using the formula:

$$\Upsilon = \{1\text{-}[(N_t - N_i)/ N_t]^{1/n} \} \times 100$$

where Υ is % infection level, N_t is no. of groups of 10 leaves tested, N_i is no. of groups testing positive for AMV, and n is number of leaves in a group (n = 10).

Variation among virus isolates

Five isolates of AMV were selected for further characterisation based on symptoms on the hosts (Table 1) and inoculated plants of *Chenopodium amaranticolor.* The isolates were each inoculated to *C. amaranticolor* and systemically infected leaves were used to inoculate plants of *N. glutinosa* for maintenance of the virus for further studies.

Seeds of some species of Amaranthaceae, Chenopodiaceae, Fabaceae and Solanaceae were grown on steam-pasteurised forest soil in an aphid-proof screenhouse. Leaves of 5–10 young seedlings per species were dusted with celite (600 mesh) and inoculated mechanically with each isolate. The inoculum was obtained by grinding infected leaves of *N. glutinosa* in 0.05M phosphate buffer pH 7.2, containing 0.1% (w/v) Na_2SO_3 at the rate of 1g tissue per 4ml buffer. Plants were observed daily for local and systemic symptoms. Thirty days after inoculation, composite samples from leaves developing after inoculation were tested for AMV serologically by ACP-ELISA to confirm systemic infection.

Table 1. Origin and description of alfalfa mosaic virus isolates used for variation studies.

AMV isolate	Source	Symptoms in host
Ms6984/3	*Medicago sativa* accession 6984[1]	Mild mottle
Ms 6984/5	*Medicago sativa* accession 6984	Mild mottle
Ma 397	*Macroptilium atropurpureum* accession 397	Bright yellow mosaic, leaf deformation
Nw 6761	*Neonotonia wightii* accession 6761	Bright yellow mosaic, leaf deformation
Nw 9979	*Neonotonia wightii* accession 9979	Bright yellow mosaic

[1]Accession numbers are ILRI Genebank entries.

Comparative serological detection of the virus isolates by ELISA was studied in *Glycine max, Gomphrena globosa* and 2 cultivars of *N. tabacum*. For each of the plants, young seedlings of similar age were inoculated mechanically with each of the AMV isolates. Three weeks after inoculation, leaves at about the same developmental stage were used to test for AMV by ACP-ELISA. Leaf samples extracted in 0.05M carbonate buffer containing 0.1% Na_2SO_3 (1:10 w/v) were each applied to quintuplate wells and each test was repeated 3 times. The absorbance at 405nm (A_{405}) was determined 60 min after application of substrate.

Results

Occurrence of AMV in field and seed

Alfalfa mosaic virus was detected in 24 forage legume species (Table 2). Persistent chlorosis was a common symptom associated with AMV-infected plants in the field. Infected plants of *M. sativa* and *Cajanus cajan* showed mottle symptoms. In some species, like *Desmanthus virgatus* and *M. truncatula*, infected plants showed reduced vigour in the field. The virus was not detected from the following species tested: *Aeschynomene americana, Arachis pintoi, Centrosema pubescens, Desmodium intortum, D. uncinatum, Erythrina burana, Flemingia macrophylla, Gliricidia sepium, Lotononis* spp., *Lotus corniculatus, L. uliginosus, Lupinus albus, L. angustifolius, L. mutabilis, Macroptilium heterophyllum, Medicago littoralis, Pisum arvense, P. sativum, Pueraria phaseoloides, Sesbania hirtistyla, Stylosanthes guianensis, Trifolium decorum, T. quartinianum, T. pratense, T. repens, T. semipilosum, Vicia benghalensis, V. villosa* and *Vigna unguiculata.*

Seed-borne infection varied from zero percent in *N. wightii* acc. 9979 and *Vigna parkeri* to 12.9% in *Chamaecrista rotundifolia*. Seedlings from the growing-on test showed mild chlorosis or remained symptomless.

Infectivity studies of five AMV isolates

The reaction of various test plants to inoculation with 5 isolates of AMV is shown in Table 3. *C. amaranticolor, Gomphrena globosa, P. sativum, Nicotiana tabacum, Neonotonia wightii, Macroptilium atropurpureum* and *Medicago sativa* cv. Hunter River were infected systemically with all the isolates. Only Ms 6984/3 infected *P. vulgaris* cv. Top Crop systemically while all others caused only local necrosis. All isolates except Ms 6984/3 incited necrotic local lesions in at least one of the *V. unguiculata* accessions tested. None of the isolates infected *P. acutifolius* systemically although they caused local necrosis except for NW 6761 which did not develop symptoms.

Absorbance values in virus detection by ELISA varied with the host plant and virus isolate (Table 4). The absorbance values were generally high in *Nicotiana tabacum* cv. White Burley and low in *Gomphrena globosa* for all the virus isolates. The absorbance value for Ms 6984/5 was about twice that for Ms 6984/3 in *Glycine max* while the reverse was true in *Gomphrena globosa*. Similar variation in absorbance values were observed between the two *Neonotonia wightii* isolates in *Nicotiana tabacum* cv. White Burley and *Gomphrena globosa.*

Table 2. Detection of alfalfa mosaic virus in field plants and seed samples of some forage legumes.

Plant	Field detection[1]	Level of seed infection (%)[3]
Aeschynomene falcata cv. Bargoo	+	6.70
Centrosema pascuorum cv. Cavalcade	+	1.44
Cajanus cajan	+	—
Chamaecrista rotundifolia	+	12.94
Desmanthus virgatus	+	2.21
Desmodium intortum cv. Greenleaf	+	0.30
Lablab purpureus (*Dolichos lablab*)	+	1.53
Leucaena leucocephala cv. Cunningham	+	0.84
Lupinus angustifolius cv. Warrah	–	0.62
Macroptilium atropurpureum	+	1.53
Macroptilium lathyroides	+	9.24
Macrotyloma axillare cv. Archer	+	1.11
Medicago sativa cv. Hunter River	+	0.71
Medicago truncatula cv. Jemalong	+	1.11
Melilotus alba	–	1.74
Neonotonia wightii cv. Tinaroo	+	0.31
Neonotonia wightii acc. 9979	+	0.00
Sesbania sesban	+	3.13
Solanum nigrum[2]	+	—
Stylosanthes scabra cv. Seca	+	0.57
Trifolium calocephala	+	—
Trifolium steudneri	+	2.84
Trifolium tembense	+	3.10
Vicia dasycarpa cv. Lana	+	0.59
Vicia sativa	+	1.89
Vigna parkeri	+	0.00

[1]+ = AMV detected in field samples; – = samples not tested.
[2]Solanaceous weed growing near alfalfa plot.
[3]% infected seeds are as determined by the method of Gibbs and Gower (1960).

Table 3. Reaction of some test plants to 5 isolates of alfalfa mosaic virus.

Test plant	Virus isolate				
	Ms 6984/3	Ms 6984/5	Nw 6761	Nw 9979	Ma 397
Chenopodium amaranticolor	$L_{NL}S_{CF,LD}$[1]	$L_{NL}S_{CF}$	L-S_{CF}	$L_{NL}S_{CF}$	L-S_{+}
Glycine max	L-S_{M}	L-S_{+}	L-S-	L-S-	L-S_{M}
Gomphrena globosa	L-S_{+}	L-S_{+}	L-S_{+}	L-S_{+}	$L_{NL}S_{NL}$
Macroptilium atropurpureum	L-S_{+}	L-S_{+}	L-S_{+}	L-S_{+}	L-S_{M}
Medicago sativa	L-S_{M}	L-S_{M}	L-S_{+}	L-S_{+}	L-S_{+}
Neonotonia wightii	L-S_{M}	L-S_{M}	L-S_{M}	L-S_{M}	L-S_{+}
Nicotiana tabacum					
cv. Samsun NN	L-S_{+}	L-S_{+}	L-S_{+}	L-S_{+}	L-S_{+}
cv. White Burley	L-S_{+}	L-S_{+}	L-S_{+}	L-S_{+}	L-S_{+}
Phaseolus acutifolius	L_{NL}S-	L_{NL}S-	L-S-	L_{NL}S-	L_{NL}S-
P. vulgaris cv. Top Crop	L-S_{M}	L_{NL}S-	L_{NL}S-	L_{NL}S-	L_{NL}S-
Pisum sativum	L-S_{M}	L-S_{M}	$L_{NL}S_{VN}$	L-S_{+}	L-S_{+}
Vigna unguiculata					
ILRI No 6790	L-S-	L-S-	L-S-	L-S-	L_{NL}S-
9332	L-S-	L_{NL}S-	L-S-	L-S-	L_{NL}S-
9334	L-S-	L_{NL}S-	L-S-	L-S-	L_{NL}S-
11971	L-S-	L_{NL}S-	L_{NL}S-	L_{NL}S-	L_{NL}S-

[1]L =Local symptoms; S = Systemic symptoms; - = No symptoms; no systemic infection; + = Symptomless systemic infection as confirmed by ELISA; CF = Chlorotic fleck; LD = Leaf deformation; M = Mosaic/mottle; NL = Necrotic lesions; and VN = Veinal necrosis.

Table 4. Detection of five alfalfa mosaic virus isolates from different test plants by ELISA.

Antigen	Test plant							
	Nicotiana tabacum cv. White Burley		*N. tabacum* cv. Samsun NN		*Glycine max*		*Gomphrena globosa*	
	A_{405}[1]	CV	A_{405}	CV	A_{405}	CV	A_{405}	CV
Ma 397	1.902	3.9	1.907	4.2	0.965	6.2	0.894	4.9
Ms 6984/3	1.490	1.3	1.983	2.6	0.748	7.2	0.869	5.5
Ms 6984/5	1.038	2.8	1.932	4.4	1.432	15.0	0.339	2.4
Nw 6761	1.122	2.6	1.838	8.3	0.026	5.1	0.479	7.5
Nw 9979	2.000	0.0	0.616	3.7	0.027	2.9	0.980	5.4
Healthy	0.021	3.4	0.027	6.3	0.022	7.2	0.019	3.3

[1] A_{405} = Absorbance values at 405nm recorded 60min after application of substrate. Values are means of 15 replicates (5 per plate, 3 plates).

Discussion

The survey data show that AMV occurs commonly among forage legumes. Of the 24 species in which the virus was detected, *Aeschynomene falcata, Centrosema pascuorum, Chamaecrista rotundifolia, Desmanthus virgatus, Desmodium intortum, Leucaena leucocephala, Macroptilium atropurpureum, M. lathyroides, Macrotyloma axillare, Medicago truncatula, Neonotonia wightii, Sesbania sesban, Stylosanthes scabra, Trifolium calocephala, T. steudneri, T. tembense* and *Vigna parkeri* are recorded for the first time based on available world literature (Jaspars and Bos 1980; Edwardson and Christie 1986; Brunt *et al.* 1990). Most of these are tropical species for which information on virus diseases is lacking (Morales 1994). In Ethiopia, Mohamed-Saleem and Berhe (1994) noted the prevalence of virus-like symptoms on *N. wightii*, but these were not attributed to any particular virus. AMV was not detected in the following species that are known to be hosts of the virus: *Lotus corniculatus, Lupinus albus, L. angustifolius, L. mutabilis, Pisum arvense, P. sativum, Trifolium pratense, T. repens, T. semipilosum, Vicia villosa* and *Vigna unguiculata.* However, *P. sativum* and *V. unguiculata* were infected experimentally.

Solanum nigrum is a common weed in Ethiopia. Samples tested in this study were from stunted plants with severe mosaic growing near lucerne plots. It was not clear if this weed was the source of infection for lucerne or *vice versa*. However, the importance of this weed in the epidemiology of AMV in this area should not be underestimated. The observation here is different from that of McKirdy and Jones (1994), where systemic infection of *S. nigrum* was not achieved by mechanical inoculation with the EW strain of AMV. This may be accounted for by virus strain differences.

The level of seed-borne inoculum was generally low. This is consistent with earlier reports (Edwardson and Christie 1986; Pathipanawat *et al.* 1995). The low level of seed-borne infection makes virus elimination through intensive roguing in the screenhouse feasible with minimal erosion of genetic variation of each accession. However, this management technique may have limited application where infected seedlings remain symptomless as was observed in the growing-on test. AMV was not detected in seeds of *V. parkeri* and *N. wightii* acc. 9979 even though they were infected in the field. This could be an indication of resistance to seed transmission but further studies are needed to confirm this.

The reaction of test plants to inoculation with the 5 AMV isolates shows that there is some variation among the prevalent isolates. This was further confirmed by variation in ELISA absorbance values. The absorbance value gives an indication of virus concentration in the test sample. Virus multiplication in a host is a strain-specific character and was shown to vary among the AMV isolates in this study. The isolates can be divided broadly into 2 strains, *viz.* those that cause necrotic local lesions on *V. unguiculata* and those that do not. The isolate Ms 6984/3 is in the latter group while the remaining 4 isolates fall in the former. Systemic infection of *P. vulgaris* cv. Top Crop may be used as a confirmatory test for this designation. There are some minor differences among the isolates causing necrotic local lesions in cowpea. Using the test cowpea accessions and other differential hosts, the individual isolates can be distinguished. Numerous strains of AMV have been classified, based on pathogenicity, host range and/or symptomatology differences (Jaspars and Bos 1980; Brunt *et al.* 1990). Kaiser and Robertson (1976) earlier identified 2 strains in east Africa from potato and lucerne. The present work further confirms their finding that the AMV population in east Africa is heterogeneous.

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