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Occurrence, Distribution and Properties of Alfalfa Mosaic Virus

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حدوث مرض تبرقش البرسيم الفيروسي و توزعه وصفاته

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خلاصة: تم تسجيل مرض تبرقش البرسيم (القت) الفيروسى (AlfMV) في سلطنة عمان على ٢١ عائلاً نباتياً تتضمن ٤ محاصيل حقلية و ١٤ محصول خضار ونبات زينة واحد وعائلين جديدين من الحشائش، موزعه بين ٩ عوائل نباتية. تم تأكيد التعرف على وجود الفيروس اعتمادا على خصائصه البيولوجية والسيرولوجية (ELISA) وبعض الخصائص الفيزيائية. احتوت الأوراق و السيقان و منطقة التاج في النباتات المصابة على تركيز عالٍ من الفيروس. وقد تم نقل المرض بصورة غير مباشرة بواسطة حشرة من القطن (*Aphis gossypii*). ويمكن أن تعزى النسبة العالية من الإصابة و توزيع المرض على مستوى السلطنة إلى العوامل التالية: المدى العوائلي الواسع بما فيها تلك النباتات التي تعمل كمخزون طبيعي للفيروس و انتقال المرض بواسطة البذور (نسبة الإصابة ٢-٣٦ %) و الانتقال بواسطة الحشرات. إن الفيروس المعزول له نقطة تخفيف نهائية بين ١X ١٠$^{-٣}$ الى ١X ١٠$^{-٤}$ ونقطة تثبيط حرارية بين ٦٥-٦٧ ^{o}م وفترة نشاط لعدة أيام عند زراعة الفيروس مختبريا. وبناء على ما تقدم فإنه يمكن الاستنتاج بأن الفيروس المعزول يشابه سلالة فيروس تبرقش البرسيم (AlfMV-S strain).

ABSTRACT: Alfalfa Mosaic Virus (AlfMV) was recorded on 21 hosts comprising of four field crops, 14 vegetables, one ornamental plant and two new weed species (*Heliotropium europaeum* and *Ammi majus*) belonging to nine families. The virus was identified and confirmed on the basis of its biological, serological (ELISA) and physical properties. The leaves, stem and crown from systemically infected alfalfa plant contained high concentration of the virus. It was nonpersistently transmitted by cotton aphids (*Aphis gossypii*). The wide host range, including virus reservoirs, seed-borne infection and insect transmission account for high incidence and distribution of AlfMV in the country. The virus isolate had a dilution end point between 1 x 10^{-3} to 1 x 10^{-4}, 65-67 °C thermal inactivation point and a few days in-vitro longevity and appears to be similar to the AlfMV-S strain.

Keywords: Alfalfa mosaic virus, host range, seed transmission, aphids.

Alfalfa (*Medicago sativa* L.) is an important perennial fodder crop in the Sultanate of Oman, occupying about 11,350 ha which represents 15.4% of cultivated area in the country (MAF, 1997). The crop in the field lasts as long as 10 years or more. This feature favors the development and build up of inoculum potential of several diseases caused by soil-borne pathogens, viruses and phytoplasmas to which alfalfa and legume hosts are susceptible.

Thirty-one viruses representing 13 virus groups are reported to systemically infect alfalfa in the world, but alfalfa mosaic virus (AlfMV) is the most common, important and widespread (Hull 1969, Regenmortel and Pink, 1981; Paliwal 1982).

The virus has been reported to infect about 400 hosts in 50 families. It occurs naturally in many herbaceous and woody plant species and remains symptomless under some conditions (Thornberry, 1966; Frosheise, 1969; Beczner and Lehoczky, 1981; Brunt *et al*., 1990). Several plants of the families Compositae, Fabaceae, Solanaceae and Umbelliferae are particularly and variably infected by AlfMV (Jasper and Bos, 1980; Brunt *et al*. 1990). In alfalfa, it induces severe symptoms, affects nodulation, reduces vigor and survival, and causes significant reduction in yield (Tu and Holmes, 1980; Bailiss and Ollennu, 1986; Hiruki and Miczynski, 1987). In view of the frequent and widespread occurrence of AlfMV, our studies were conducted on the identity, host range, transmission and epidemiological aspects of the virus using Oman as the case study area.

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TABLE 1

Herbaceous hosts naturally infected by AlfMV.

Host Plant	Common Name	Symptoms**	ELISA Reaction
FAMILY APOCYNACEAE			
Catharanthus roseus	Periwinkle	Mo, Mot. Chl	++
FAMILY BORAGINACEA			
Heliotropium europaeum	Heliotrope	Severe and bright, Mo, Chl. Flecks	+++
FAMILY CHENOPODIACEAE			
Spinacea oleracea	Spinach	Mild Mo and Mot.	+
FAMILY COMPOSITAE			
Carthamus tinctorius	Safflower	Mild Mo, Chl.	++
Helianthus annuus	Sunflower	Mild Mo, Mot	++
Lactuca sativa	Lettuce	Mo, Mot	++
FAMILY CUCURBITACEAE			
Citrullus lanatus	Watermelon	Mo, Mot, Chl. St.	+++
Cucumis melo	Sweetmelon	Chl.	+
Cucurbita pepo	Squash	Mild Mot.	+
FAMILY FABACEAE			
Cicer arietinum	Chickpea	Chl, Nec, St	+++
Medicago sativa	Alfalfa	Mo, Mot, Mal.	+++
Pisum sativum	Pea	Nec. St, Death	+++
FAMILY MALVACEAE			
Abelmoschus esculentus	Okra	Chl Flecks	++
FAMILY SOLANACEAE			
Capsicum annuum	Pepper	Mo, Chl	+++
Capsicum frutescens	Pepper	Mo, Chl.	+
Lycopersicon esculentum	Tomato	Mo, Chl Nec.	++
Solanum melongena	Eggplant	Mo, Chl.	++
Solanum tuberosum	Potato	Bright Mo, Calico, tuber nec.	++++
FAMILY UMBELLIFERAE			
Ammi majus	Ammi	Bright yellow Mo	++++
Coriandrum sativum	Coriander	Mo, Chl	+++
Daucus carota	Carrot	Mo, Chl.	++++

**Chl - chlorosis, Mal.- malformation, Nec.-Necrosis, Mo – Mosaic, Mot.- Mottling, St.- stunting.
ELISA reaction as OD values at 490 nm (+ weak 0.2, ++ moderate 0.3, +++ moderately strong 0.4, and ++++ strong 0.5 and above).

TABLE 2

Detection of AlfMV in different parts of a systemically-infected alfalfa plant.

Part Tested	ELISA Test	
	Reaction	OD 490 nm
Leaves	Strong	0.480
Stems	Strong	0.518
Leaflets	Strong	0.465
Crown	Strong	0.462
Bark	Moderate	0.215
Root	Moderate	0.300
Root hairs	Moderate	0.295
Wood	-	0.052
Control positive	Strong	0.561
Healthy leaves	-	0.051
Buffer	-	0.045

- No reaction.

Materials and Methods

SURVEYS AND COLLECTION OF SPECIMENS: About 250 farms located in different regions of Oman (Batinah, Dakhliya, Dhahira, Sharqiya and Dhofar) were surveyed at appropriate times over a protracted period (1993-1997). Field crops and vegetables growing close or in vicinity of alfalfa were examined. Host plants with characteristic or suspected symptoms of AlfMV were collected in sterilized polythene bags and stored at 4 °C until processed (Table 1). All collections were analyzed by ELISA in the laboratory. The material collected was cleaned, sorted out and divided into three portions. One portion was finely chopped, vacuum dried over anhydrous calcium chloride in a desiccator for 48 hours and preserved in glass tubes in the freezer (Walkey, 1992). The second part was used for ELISA tests and the third one for mechanical inoculation, insect transmission and sap properties. In order to determine virus concentration in the host, systemically infected individual alfalfa plants were maintained, samples were collected from different parts of the plants and one composite sample of each part was analyzed through ELISA (Table 2).

HOST RANGE, MECHANICAL INOCULATION AND SYMPTOMATOLOGY: Test plants (Table 3) were raised in an insect-free growth room maintained at 25-27 °C with 14-hour artificial light. Inoculum was prepared by grinding infected tissue in 0.02 M phosphate buffer having pH 7.2, in a pestle mortar (1gm/2ml) and squeezed through two layers of muslin cloth. The leaves of the test plants were lightly dusted with 400- mesh carborundum and inoculated mechanically with the infective extract. The plants were immediately washed with tap water to remove superfluous inoculum and maintained in the growth room for four weeks for symptom expression (Bos, 1970). Appropriate controls were also included.

SEED TRANSMISSION: Heavily infected alfalfa plants were marked, maintained and allowed to mature. The seeds were harvested at maturity. Seed samples were also collected from different regions and sources. Controls consisted of breeders' seed and seeds collected from healthy plants of the same age. These were germinated

TABLE 3

Reaction of some plant species following mechanical inoculation with AlfM-infective sap.

Plant Species	Common Name	Reaction**
Arachis hypogaea	Peanut	Mo, Mot, Chl
Cajanus cajan	Pigeon pea	Nec, NLL
Chenopodium quinoa	Chenopodium	Chl, Nec, LL, Fl
Ch. amaranticolor	"	Chl. NLL Sys, Fl.
Glycine max	Soybean	Mo, Chl, Nec, St
Lens esculenta	Lentil	Mo, Chl, St
Medicago sativa	Alfalfa	VC, Mo, St, Mal
Nicotiana tabacum	Tobacco	VC, VB, LL, Mo, Chl
N. clevelandii	Tobacco	VC, LL, Mo, Chl
N. glutinosa	Tobacco	LL, Chl
N. rustica	Tobacco	Mo, Mot, Chl
Phaseolus lunatus	Lima bean	NLL, Chl
Phaseolus vulgaris	Bean	Chl, Nec, LL, Mo, Mot
Pisum sativum	Pea	LL, Nec, wilt
Vicia faba	Broad bean	NLL, Mo, Chl, St, Nec
Vigna mungo	Black gram	NLL, Chl
V. radiata	Green gram	NLL, Chl
V. unguiculata	Cowpea	NLL, Chl

** Mo = Mosaic; Mot = Mottling; Nec = Necrosis; NLL = Necrotic Local Lesion; Chl = Chlorosis; Fl = Flecks; Sys = Systemic; St = Stunting; VC = Vein Clearing; VB = Vein Banding; Mal = Malformation.

and 4-6 months old seedlings were tested for percent seed transmission of AlfMV (Walkey, 1992) by ELISA.

INSECT TRANSMISSION: Cotton aphid (*Aphis gossypii* Glov.) was used as a vector to transmit AlfMV (Zitter, 1977). Non-viruliferous aphids were reared on cotton at 25°C for one month, batches of young aphids were removed from the leaf by using a brush, starved for about one hour and transferred to AlfMV-infected alfalfa plants and allowed to feed for 2-5 minutes for virus acquisition. These were gently removed and placed on healthy alfalfa seedlings for inoculation feeding. All the aphids on the plants were killed by spraying with an insecticide and plants kept in a cage for one month for symptom expression.

ELISA TESTS: All samples were tested by double antibody sandwich (DAS) ELISA according to the method of Clark and Adams (1977). The reagents were obtained from Agdia, Indiana USA (Lot Nos. 0123 IgG, 0126 Peroxidase-conjugated IgG). The plates were observed visually and read in Pasteur Reader LP 300 at 490 nm.

Results

NATURAL HOST RANGE OF ALFMV: Host plants naturally infected by AlfMV in Oman are listed in Table 1. The infected plants were ELISA positive and manifested typical symptoms consisting of mosaic and mottling, streaks, chlorotic flecks, necrosis, stunting and malformations. The virus was recorded on 21 hosts consisting of four field crops, 14 vegetables, one ornamental plant and two weed species. Neinhaus (1981), Brunt *et al.* (1990) and Walkey *et al.* (1990) have reported similar hosts of AlfMV in the tropical areas. Our collection, however, included three new hosts, watermelon (*Citrullus lanatus*) and two weed species (*Heliotropium europaeum* and *Ammi majus*) which are reported for the first time.

The virus was distributed in all parts of the alfalfa plant. Leaves, stems and crowns contained the maximum concentration of the virus (Table 2).

TABLE 4

Extent of virus-infected seeds in alfalfa seed lots.

Seed Source	Infected/Examined	Infection (%)	OD value 490 nm
Infected plant	58/226	26.0	0. 473
Farmers, (Batinah)	12/650	1.8	0.370
Farmers, (Interior)	45/540	8.3	0.385
Commercial seed	51/500	10.2	0.330
Healthy plants	0/200	0.0	0.068
Breeders' seed	0/150	0.0	0.050
Controls			
Infected leaves (+ve)	-	-	0.625
Healthy leaves (-ve)	-	-	0.042
Buffer	-	-	0.047

MECHANICAL INOCULATION: Reaction of 18 test plant species following mechanical inoculation with infective alfalfa sap are given in Table 3. Results confirmed that the symptoms observed in naturally infected hosts could be reproduced in the test plants by mechanical inoculation. On the basis of systemic infection, *Nicotiana* spp. and alfalfa were selected as propagative hosts for the virus and *Chenopodium* spp. and *Phaseolus vulgaris* were the best local lesions hosts for infectivity assays. *Brassica* spp. *Datura stramonium* and *Petunia hybrida* were infected by AlfMV and gave negative reactions in ELISA.

SEED TRANSMISSION: Seeds harvested from systemically infected alfalfa plants were germinated. The seedlings in ELISA tests showed 26% infection of AlfMV (Table 4). Similarly, seed transmission of AlfMV in the farmers' samples was 2% in the Batinah region, 8% in the interior and 10% in the commercial seed stock. Brunt *et al.* (1990) have reported 50% seed transmission from individual infected plants and up to 10% in commercial seed stocks. Thus the results obtained in our study are in close conformity.

APHID TRANSMISSION: AlfMV was efficiently transmitted by *Aphis gossypii* in a non-persistent manner. Using five individuals in a batch/seedling, giving a virus acquisition period of two minutes and

same length of time as virus inoculation feeding. Symptoms of virus with aphid transmission appeared in alfalfa seedlings after 18-21 days. The infection was confirmed by ELISA.

PHYSICAL PROPERTIES: Aliquots of infective sap from alfalfa were subjected to different treatments using standard procedures suggested by Noordam (1973). The infectivity of each treatment was assayed on half leaves of *Phaseolus vulgaris*. The infective sap showed the following properties: Dilution end point (DEP) between 1×10^{-3} to 1×10^{-4}, 65-67 °C thermal inactivation point (TIP) and 3 days *in vitro* longevity at 25 °C. It was resistant to chloroform, carbon tetrachloride, buffers of high morality (phosphate, borate, citrate), and it retained infectivity in a wide pH range (3 to 10). The isolate of AlfMV present in Oman has a higher TIP than that reported by Brunt *et al.* (1990). This could contribute to its survival and adaptation under prolonged warmer conditions prevalent in the country.

Discussion

Alfalfa mosaic virus was found to be commonly present in every topographical region, therefore naturally infecting a wide range of host plants. A majority of these serve as virus reservoirs (Table 1). The host range of AlfMV is likely to be wider than reported in our study because the collection still excludes fruit and forest trees which need to be surveyed. So far, the virus was recorded on 21 hosts, and the collection includes three new or unreported hosts; two weed species (*H. europaeum* and *A. majus*) and watermelon. Typical symptoms of AlfMV were reproducible in test plants by mechanical inoculation (Table 3). This also differentiated indicator and propagative hosts for the virus. As expected, the virus was found to be seed-borne to an extent sufficient to horizontal and long-distance spread as well as for the establishment of primary inoculum. Thirteen aphid species are known to non persistently transmit and spread AlfMV in different hosts. *A. gossypii* was selected because of its close association and abundance on several host plants. It proved to be an efficient vector of AlfMV. Systemic distribution of the virus in the host and the physical properties of infective sap indicate that AlfMV is stable and well adapted to local conditions. The virus is reported to consist of large number of strains (Beczner and Lehoczky 1980, 1981; Hiruki and Miczynski, 1987). The results suggest that the strain-S of AlfMV is prevalent in Oman.

Wide distribution and frequent occurrence of AlfMV may be attributed to appreciable levels of seed transmission, a high aphid population, a wide host range, and a conventional cropping system. All these factors seem to be involved in the epidemiology of AlfMV. Traditional farming systems of growing vegetables and field crops close to alfalfa fields greatly favors the spread of the virus through aphids. According to Hiruki and Hampton (1990), an initial 11% incidence of AlfMV-infected crop in the greenhouse increased to 91% after nine cuttings within 10 months. Similarly, with prolonged life of alfalfa in the field, infection levels of AlfMV became greater for its spread to other crops and interseasonal vegetables (Walkey, 1992). Therefore, in order to check initial inoculum and to subsequently alleviate serious infections of AlfMV, use of virus-free seed should be the first step. Removal of weed hosts and virus reservoirs and separation of vegetables and field crops from the perennial alfalfa with a distance of 100 meters can be effective in reducing the infection significantly (Thresh, 1982; Walkey 1992). As alfalfa constitutes an integral part of farm life and is extremely important to the ecology, a crop improvement program including introduction of virus-resistant cultivars needs to be initiated. Crill and Hanson (1969), Crill *et al.* (1971) and Hiruki and Miczynski (1987) have reported some alfalfa cultivars which are resistant or tolerant to AlfMV.

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