OCCURRENCE, DISTRIBUTION AND SOME PROPERTIES OF ALFALFA MOSAIC ALFAMOVIRUS IN THE SULTANATE OF OMAN

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Alfalfa mosaic Alfamovirus (AMV) was widely distributed in Sultanate of Oman as 88% of the fields surveyed were found to be infected. The virus was identified on the basis of biological, serological and some physical properties, and was recorded on 21 hosts comprising of 4 field crops, 14 vegetables, 1 ornamental plant and 2 weed species; distributed in 9 botanical families. Two new hosts of AMV i.e. Heliotrope (Heliotropium europaeum) and Ammi (Ammi majus) were found. The virus was detected in all parts of systemically infected plants except wood. Seed transmission in the farmers' samples, commercial stock and seeds harvested from mechanically inoculated plants was 2 to 8%, 10.2% and 26%, respectively. The virus isolate had a dilution end point 1 x 10^-3 - 10^-4, longevity in vitro for 3 days at 25°C and thermal inactivation point of 65-67°C. Cotton aphids (Aphis gossypii) transmitted the virus in a non-persistent manner. Wide occurrence and distribution of AMV in the country is attributed to its broad host range, seed-transmission, adaptation to high temperature and abundance of insect vector.

Key words: Alfalfa mosaic alfamovirus, host range, seed transmission, cotton aphids.

INTRODUCTION

Alfalfa (Medicago sativa L.) is an important perennial fodder crop in the Sultanate of Oman, occupying about 11350 ha which represents 15.4% of cultivated area in the country (Anon., 1997). The duration of the crop in the field extends from few to 10 years or more. This feature has favoured the development and build-up of inoculum potential of several soil-borne pathogens as well as certain viruses and phytoplasmas. Among 31 viruses infecting alfalfa crop in the world, alfalfa mosaic alfamovirus (AMV) is the most common and widespread (Regenmortel and Pink 1981, Paliwal 1982). AMV induces severe mosaic and bright yellow symptoms, affects nodulation, reduces vigor and causes significant reduction in yield (Tu and Holmes 1980, Bailiss and Ollennu 1986, Hiruki and Miczynski 1987). The host range of AMV is enormously wide infecting about 400 plant species in 50 families, mostly in Compositae, Fabaceae, Solanaceae and Umbelliferae (Jasper and Bos 1980, Brunt et al. 1990). Several herbaceous and woody hosts are naturally infected and some remain symptomless under certain conditions (Frosheiser 1969, Beczner and Lehoczky 1980, Brunt et al 1990). This study was conducted to determine occurrence, distribution, natural host range, transmission and other properties of AMV in the Sultanate of Oman.

MATERIALS AND METHODS

Surveys and collection of samples: About 250 farms located in the five regions of Oman (Batinah, Dakhliya, Dhahir, Sharqiya and Dhofar) were surveyed at appropriate times (1994-1999). One hundred and ninety three field samples of alfalfa and 142 samples of some field crops, vegetables and weeds growing close or in vicinity of alfalfa fields were examined carefully. Plants with characteristic or suspected symptoms of AMV were collected in polyethylene bags and stored at 4°C until processed for ELISA and other properties. AMV-infected tissue was finely chopped, vacuum dried over anhydrous CaCl_2 in a desiccator for 48 hr and preserved at -18°C (Walkey, 1992).

Host range, mechanical inoculation and symptomatology. Test plants were raised in an insect-free growth room maintained at 25-27 ºC with 14-hour artificial light. Inoculum was prepared by grinding infected tissue (0.5g/ml) in 0.02 M phosphate buffer, pH 7.2, in a pestle and mortar and the sap squeezed through two layers of muslin cloth. The leaves of the test plants were dusted with 400-mesh carborundum powder, inoculated mechanically with the infective extract and immediately washed with tap water to remove superfluous inoculum. Plants were maintained in the growth room for about 4 weeks for symptom expression (Bos, 1970). Appropriate controls were also included.

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Seed Transmission: Mechanically inoculated alfalfa plants were maintained in an insect-free environment and seeds were harvested from these plants at maturity. Control consisted of seeds harvested from healthy plants of the same age. Seed samples of alfalfa were also collected from the farmers and commercial sources. These were germinated and the extent of seed transmission was assessed by ELISA in 2-month old seedlings (Walkey, 1992).

Insect Transmission: Cotton aphid (Aphis gossypii Glov.) was used as a vector to transmit AMV (Zitter, 1977). Non-viruliferous aphids were reared on cotton at 25 °C, batches of 5 aphids were removed by a brush, starved for about one hour and transferred to AMV-infected plants for 2-3 minutes to acquire the virus. Aphids were gently removed and placed on each of 20 healthy alfalfa seedlings for inoculation feeding and then killed by spraying with an insecticide. The plants were kept in a growth room for two months for symptom expression.

ELISA Tests: All samples were tested against monoclonal antibodies of AMV 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. Some samples were also tested against polyclonal antisera or monoclonal antibodies of BYMV, PVY, PVX, CMV, TMV and ACMV.

RESULTS

Distribution of AMV: It was found that 88% of the alfalfa fields harboured AMV infection with various categories of incidence (Figure 1). Based on ELISA test, 12% of the fields were free of infection, 17.6% had infection in traces and 3.6% were heavily infected. The remaining fields i.e. 34.2%, 20.2% and 12.43% showed clear infection ranging between 5-50%. The disease was distributed in all regions of the country (Figure 2) but Dhahira region had high disease incidence (17.8%) followed by Dakhliya (11%), Sharqiya (10%) and Batinah (8.5%). It was noted that the plants of all ages were infected but severity of disease and incidence were higher in the ratooned crop. The virus was detected in all parts of heavily infected alfalfa plants except wood, but leaves, stems and crown contained maximum virus concentration (Table 2). This suggests that alfalfa plants are the potential source of AMV infection. These results agree to those of Hiruki and Hampton (1990) who reported that with an initial 11% incidence of AMV-infected crop increased to 91% after nine cuttings within 10 months.

Natural Host Range and Mechanical Inoculation of AMV: The virus was recorded on 21 hosts consisting of 4 field crops, 14 vegetables, 1 ornamental plant and 2 weed species, all distributed in 9 families (Table 1). Two weed species (Heliotropium europaeum and Ammi majus) were recorded as new hosts of AMV. The naturally infected plants manifested typical symptoms consisting of mosaic and mottle, streaks, chlorotic flecks, necrosis, stunting and malformations. Majority of the symptoms could be reproduced in test plants by mechanical inoculations. All the plants were ELISA positive for AMV and did not react against antibodies to TMV, PVX, PVY and BYMV. However, CMV was detected in spinach, squash and sweet melon, TYLCV in tomato and OLCV in okra. Reaction of 18 test plants species following mechanical inoculation with infective alfalfa sap is given in Table 3. Most of the important species of Chenopodiaceae, Solanaceae and Fabaceae were locally and systemically infected by AMV. On the basis of systemic infection, Nicotiana spp. were selected for the propagation of the virus and Chenopodium spp. and Phaseolus vulgaris were the best local lesion hosts for infectivity assays. Plants not infected by AMV and giving negative reactions in ELISA were: Brassica spp. Datura stramonium and Petunia hybrida.

Physical Properties: Aliquots of infective sap from alfalfa were subject to different treatments using standard procedures (Noordam, 1973), and infectivity was assayed on half leaves of P. vulgaris. The infective sap had dilution end point (DEP) between 1x10^-3 to 1x 10^-4, thermal inactivation point (TIP) between 65-67°C and longevity in vitro (LIV) of 3 days at 25 °C. The virus was resistant to chloroform, carbon tetrachloride, buffers of high molarity (phosphate, borate and citrate) and retained infectivity at a wide pH range 3 to 10.

Transmission of AMV: Seeds collected from mechanically and systemically infected alfalfa plants carried AMV to an extent of 26%. Similarly, seed transmission of AMV in the farmers’ samples was about 2% in the Batinah region, 8 % in the interior and 10% in the commercial seed stock (Table 4). AMV was efficiently transmitted by cotton aphids (Aphis gossypii) in a non-persistent manner. Typical AMV-symptoms appeared on the aphid-inoculated alfalfa plants within 6-21 days. The infection was confirmed by ELISA.

DISCUSSION AND CONCLUSION

Extensive surveys of alfalfa fields, collection of large number of naturally infected plants and their positive ELISA reactions and mechanical inoculation of indicator plants revealed that AMV was the most commonly and predominantly occurring disease in the Sultanate of Oman. Majority of the plants mentioned in
Occurrence, distribution and some properties of alfalfa

Table 1. Herbaceous hosts naturally infected by AMV in Oman

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Common name</th>
<th>Symptoms**</th>
<th>Reaction to antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AMV</td>
</tr>
<tr>
<td><strong>FAMILY APOCYNACEAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catharanthus roseus (L) G. Don</td>
<td>Periwinkle</td>
<td>Mo, Mot. Chl.</td>
<td>4/6</td>
</tr>
<tr>
<td><strong>FAMILY BORAGINACEAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heliotropium europaeum L.</td>
<td>Heliotrope</td>
<td>Mo, Chl. Fl.</td>
<td>20/20</td>
</tr>
<tr>
<td><strong>FAMILY CHENOPODIACEAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinacea oleracea L.</td>
<td>Spinach</td>
<td>Mo, Mot. Chl.</td>
<td>2/6</td>
</tr>
<tr>
<td><strong>FAMILY COMPOSITAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carthamus tinctorius L.</td>
<td>Safflower</td>
<td>Mo, Chl.</td>
<td>4/6</td>
</tr>
<tr>
<td>Helianthus annuus L.</td>
<td>Sunflower</td>
<td>Mo, Mot.</td>
<td>5/6</td>
</tr>
<tr>
<td>Lactuca sativa L.</td>
<td>Lettuce</td>
<td>Mo, Mot.</td>
<td>6/6</td>
</tr>
<tr>
<td><strong>FAMILY CUCURBITACEAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrullus lanatus (Thunb) Mansf.</td>
<td>Watermelon</td>
<td>Mo, Mot.</td>
<td>2/6</td>
</tr>
<tr>
<td>Cucumis melo L.</td>
<td>Sweetmelon</td>
<td>Mo, Chl.</td>
<td>1/6</td>
</tr>
<tr>
<td>Cucurbita pepo L.</td>
<td>Squash</td>
<td>Mo, Mot.</td>
<td>2/6</td>
</tr>
<tr>
<td><strong>FAMILY FABACEAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cicer arietinum L.</td>
<td>Chickpea</td>
<td>Chl. Nec. St.</td>
<td>5/6</td>
</tr>
<tr>
<td>Medicago sativa L.</td>
<td>Alfalfa</td>
<td>Mo, Mot. Mal.</td>
<td>170/193</td>
</tr>
<tr>
<td>Pisum sativum L.</td>
<td>Pea</td>
<td>Nec. St.</td>
<td>2/6</td>
</tr>
<tr>
<td><strong>FAMILY MALVACEAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abelmoschus esculentus (L) Moench.</td>
<td>Okra</td>
<td>Chl. Fl.</td>
<td>4/6</td>
</tr>
<tr>
<td><strong>FAMILY SOLANACEAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsicum annuum L.</td>
<td>Pepper</td>
<td>Mo, Chl.</td>
<td>4/6</td>
</tr>
<tr>
<td>Capsicum frutescens L.</td>
<td>Pepper</td>
<td>Mo, Chl.</td>
<td>2/6</td>
</tr>
<tr>
<td>Lycopersicon lycopersicum (L) Karst.</td>
<td>Tomato</td>
<td>Mo, Chl, Nec.</td>
<td>5/6</td>
</tr>
<tr>
<td>Solanum melongena L.</td>
<td>Eggplant</td>
<td>Mo, Chl.</td>
<td>5/6</td>
</tr>
<tr>
<td>Solanum tuberosum L.</td>
<td>Potato</td>
<td>Mo, Nec.</td>
<td>10/10</td>
</tr>
<tr>
<td><strong>FAMILY UMBOUILLIFERAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammi majus L.</td>
<td>Ammi</td>
<td>Mo</td>
<td>10/10</td>
</tr>
<tr>
<td>Coriandrum sativum L.</td>
<td>Coriander</td>
<td>Mo, Chl.</td>
<td>5/6</td>
</tr>
<tr>
<td>Daucus carota L.</td>
<td>Carrot</td>
<td>Mo, Chl.</td>
<td>2/6</td>
</tr>
</tbody>
</table>

** Chl= chlorosis, Mal.= malformation, Nec.=Necrosis, Mo= Mosaic Mot.= Mottling, St.= stunting

Table 2. Detection of AMV in different parts of systemically-infected alfalfa plant.

<table>
<thead>
<tr>
<th>Part tested</th>
<th>Reaction</th>
<th>ELISA Test</th>
<th>OD at 490 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leave</td>
<td>Strong</td>
<td>-</td>
<td>0.480</td>
</tr>
<tr>
<td>Stems</td>
<td>Strong</td>
<td>-</td>
<td>0.518</td>
</tr>
<tr>
<td>Leaflets</td>
<td>Strong</td>
<td>-</td>
<td>0.465</td>
</tr>
<tr>
<td>Crown</td>
<td>Strong</td>
<td>-</td>
<td>0.462</td>
</tr>
<tr>
<td>Bark</td>
<td>Moderate</td>
<td>-</td>
<td>0.215</td>
</tr>
<tr>
<td>Root</td>
<td>Moderate</td>
<td>-</td>
<td>0.300</td>
</tr>
<tr>
<td>Feeder roots</td>
<td>Moderate</td>
<td>-</td>
<td>0.295</td>
</tr>
<tr>
<td>Wood</td>
<td>-</td>
<td>-</td>
<td>0.052</td>
</tr>
<tr>
<td>Control</td>
<td>Strong</td>
<td>-</td>
<td>0.561</td>
</tr>
<tr>
<td>Positive</td>
<td>-ve</td>
<td>-</td>
<td>0.051</td>
</tr>
<tr>
<td>Healthy leaves</td>
<td>-ve</td>
<td>-</td>
<td>0.045</td>
</tr>
<tr>
<td>Buffer</td>
<td></td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Reaction of some plant species following mechanical inoculation with AMV.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Common name</th>
<th>Reaction**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachis hypogaea L.</td>
<td>Peanut</td>
<td>Mo, Mot, Chl.</td>
</tr>
<tr>
<td>Cajanus cajan (L) Millsp.</td>
<td>Pigeon pea</td>
<td>Nec. LL</td>
</tr>
<tr>
<td>Chenopodium quinoa Willd.</td>
<td>Chenopodium</td>
<td>Chl, Fl.</td>
</tr>
<tr>
<td>Ch. amaranthicolor C&amp;R.</td>
<td>Chenopodium</td>
<td>Chl. Fl.</td>
</tr>
<tr>
<td>Glycine max (L) Millsp.</td>
<td>Soybean</td>
<td>Mo, Chl, Nec, St.</td>
</tr>
<tr>
<td>Len culinairs Medik.</td>
<td>Lentil</td>
<td>Mo, Chl. St.</td>
</tr>
<tr>
<td>Medicago sativa L.</td>
<td>Alfalfa</td>
<td>VC, Mo, St. Mal.</td>
</tr>
<tr>
<td>Nicotiana clevelandii A. Gray</td>
<td>Tobacco</td>
<td>LL</td>
</tr>
<tr>
<td>N. glutinosa L.</td>
<td>Tobacco</td>
<td>LL</td>
</tr>
<tr>
<td>N. rustica L.</td>
<td>Tobacco</td>
<td>Mo, Mot, Chl</td>
</tr>
<tr>
<td>N. tabacum L.</td>
<td>Tobacco</td>
<td>LL</td>
</tr>
<tr>
<td>Phaseolus lunatus L.</td>
<td>Lima bean</td>
<td>VC, VB, Chl.</td>
</tr>
<tr>
<td>Phaseolus vulgaris L.</td>
<td>Bean</td>
<td>Mo, Mot. Chl.</td>
</tr>
<tr>
<td>Vicia faba L.</td>
<td>Broad bean</td>
<td>NLL</td>
</tr>
<tr>
<td>V. radiata (L) Wilczek.</td>
<td>Green gram</td>
<td>NLL</td>
</tr>
<tr>
<td>V. unguiculata (L) Walp.</td>
<td>Cowpea</td>
<td>NLL</td>
</tr>
</tbody>
</table>

**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

Table 4. Percent seed transmission of AMV in different seed samples.

<table>
<thead>
<tr>
<th>Seed source</th>
<th>Number of seedlings</th>
<th>Infection (%)</th>
<th>Average OD values at 490 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected plant</td>
<td>226</td>
<td>58</td>
<td>26.0</td>
</tr>
<tr>
<td>Farmers, (Batinah)</td>
<td>650</td>
<td>12</td>
<td>1.8</td>
</tr>
<tr>
<td>Farmers, (Interior)</td>
<td>540</td>
<td>45</td>
<td>8.3</td>
</tr>
<tr>
<td>Commercial seed</td>
<td>500</td>
<td>51</td>
<td>10.2</td>
</tr>
<tr>
<td>Healthy plants</td>
<td>200</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Breeders’ seed</td>
<td>150</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected leaves (+ve)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy leaves (-ve)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffer (-ve)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 are known to be the host of AMV (Brunt et al., 1990), except two weed species (H. europaeum and A. majus) which represent new records. Although legume production in Oman was restricted to few species, majority of the host plants infected could serve as virus reservoir. Host range of AMV could be extended in future because our collection did not include fruit and forest trees many of which are susceptible to AMV (Jasper and Bos, 1980). The seed transmission played an important role in the establishment of primary infection and horizontal spread of AMV. As expected, AMV was seed-borne to an extent of 26% from systemically infected plants, and 2 to 10% in seed samples collected from other sources (Table 4). The exchange of infected seed by the farmers, a common feature in the country, may account for recurrent incidence of AMV in some regions. Brunt et al. (1990) reported 50% seed transmission of AMV in mechanically inoculated plants and up to 10% in commercial seed stocks. The variation found in this study may be due to virus strain, host genotype and time of infection. Thirteen aphid species are known to vector AMV in a non-persistent manner. We selected A. gossypii because of its close association and abundance on several hosts including alfalfa and it proved to be a successful vector of AMV. The virus is...
Figure 1. Incidence of AMV based on field symptoms and ELISA in Oman
Figure 2: % Incidence of AMV in various regions of Oman
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reported to consist of a large number of strains (Beczner and Lehoczky 1980, Hiruki and Miczynski 1987) which are placed in monotypic genus Alfamovirus. Based on host reaction and physical properties, the local isolate has close resemblance with common strain of AMV. Physical properties of infective sap suggest that AMV is a stable virus and is well adapted to local conditions for its survival, especially to prolonged warmer conditions in the country.

Wide distribution of AMV in Oman might be attributed to several epidemiological factors such as wide host range high aphid population, seed transmission, and ratooning of the crop. Traditional farming system of growing vegetables and field crops close to alfalfa fields greatly favors the spread of the virus through aphids. Similarly, with prolonged life of alfalfa in the field, infection levels of AMV can become greater for its spread to other crops and interseasonal vegetables (Walkey, 1992). Therefore, use of virus-free seed is essential to check primary infection. Removal of weed hosts and separation of vegetables and field crops from the perennial alfalfa with a distance of 100 meters might be expected to reduce the infection significantly (Thresh 1982, Walkey 1992). As alfalfa constitutes an integral part of farm life and is extremely important to the ecology of Oman, a crop improvement program including selection of virus-resistant cultivars needs to be initiated (Crill et al. 1971, Hiruki and Miczynski 1987).

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