Pak. J. Agri. Sci., Vol. 43(1-2), 2006

SOME BIOLOGICAL, SEROLOGICAL AND PHYSICAL PROPERTIES OF TOBACCO MOSAIC VIRUS (TMV) FROM THE SULTANATE OF OMAN

S.M. Mughal*, S. Zidgali and A.R. Matrooshi
Department of Crop Protection,
Agricultural Research Centre Rumais, Sultanate of Oman.

TMV was isolated from plant samples collected from tobacco fields and identified and characterized on the basic of biological, serological and physical properties. The virus isolate infected 23 plant species in 5 botanical families, had DEP of 1x 10^{-7}, TIP between 85-88°C and longevity *in vitro* of 30 days at 25°C. Fresh and desiccated tissue was ELISA positive. Purified virus preparations were infectious, opalescent and absorbed UV spectrum which was minimum at 242 nm and maximum at 260 nm. Antiserum produced against TMV in a rabbit showed a homologous titre of 1:512 in gel immunodiffusion test and 1:2048 in tube precipitin test. Numerous, rod shaped unaggregated virus particles, with a modal length of 300 nm, were observed under EM. On the basic of hosts range, smptomatology and serological properties, the TMV isolate resembled very closely to common strain of TMV.

Keywords: TMV, ELISA, virus characterization, common strain.

INTRODUCATION

Tobacco mosaic virus (TMV), type member of the genus Tobamovirus (Bos 1999, Matthews 2004) is a ubiquitous and serious virus disease world wide. Historically, TMV is very important because it was the first plant virus to be recorded, and is associated with many advancements in plant virology and molecular biology. It has enjoyed several "Firsts" to its credit during the course of its studies (Regenmortel 1982, Fraenkel-Conrat *et al.*1986, Agrios 2004).TMV has been studied intensively and extensively and has contributed greatly to our understanding of plant and animal viruses. Pathologically, TMV is important because it causes serious diseases, probably attacking and damaging a great variety of plants than any other virus. About 200 hosts across 30 botanical families, including many hosts of economic importance, are infected by TMV in the world (Regenmortel, 1982). The virus is exceptionally stable and can withstand adverse conditions better than the hosts. It is sap transmissible, spreads by contact, has no known natural vector and survives for a long time in the infected tissue, sap, plant debris, contaminated seed and even in cigarettes. TMV exists in the form of numerous strains and several mutants are known which make it a rich diversity (Hennig and Wittmann, 1972).

Meager information is available on the occurrence and identification of plant viruses in the Sultanate of Oman. Waller and Bridge (1978) published a comprehensive account of fungal and nematode diseases present in Oman but made no record of virus diseases. Hassan *et al.* (1985) also overlooked virus disease in the disease surveys of vegetables and field crops. Therefore, a plant disease survey program, including identification and characterization of vial diseases, was initiated for the first time in the country (Zidgali and Mughal, 1993). This paper reports occurrence and some properties and characteristics of TMV isolated from field collected samples in Oman.

MATETRIAL AND METRHOD

Virus Culture: Tobacco fields were surveyed at several places, particularly at Shinas and adjacent areas in northern Oman and virus infected plant samples were collected in polythene bags and stored at 4°C until processed. All the plants were raised in an insect-free glasshouse maintained at 25-27°C with 12-hours fluorescent illumination. Growth medium consisted of peat and soil mixture (1:1) and contained in plastic pots 10 cm in diameter. Mosaic-infected leaves were processed and the virus was passed to *Nicotiana glutinosa* by frequent transfers, serologically checked for its freedom from other viruses especially tomato mosaic , cucumber mosaic and PVX, and pure isolate of the virus was maintained and propagated in *N. tabacum* cv. White Burley. Inoculum was prepared by grinding infected tissue in a pestle and mortar in presence of 0.02 M phosphate buffer, pH 7.2 (1g/ml). Test plant species were lightly dusted with 600-mesh carborundum powder, inoculated mechanically with infective sap or virus preparation with forefinger, then rinsed immediately with tap water to remove superfluous inoculum. Plants were observed for symptom development for 10-15 days (Bos, 1970,

*Senior author was on deputation from the Pakistan Agricultural Research Council, Islamabad. Present address: Department of Plant Pathology, University of Agriculture, Faisalabad.

Bock 1972). Infectivity assays were made on half leaves of *Chenopodium amaranticolor*.

***In-vitro* properties:** Physical properties of infective sap, e.g. dilution end point (DEP), thermal inactivation point (TIP), longevity *in vitro*, resistance to chemicals, were determined according to standard methods described by Bock (1972) and Noordam (1973).

Virus Purification: Systemically infected leaves of *N. tabacum* were homogenized in 0.2 M sodium citrate buffer, pH 7.8, containing 0.15% thioglycollic acid (1g/2 ml). The homogenate was strained through two layers of muslin cloth and the expressed sap was emulsified with one half volumes each of chloroform and carbon tetrachloride. The emulsion was broken by centrifugation at 10K for 15 minutes and the buffer phase was recovered. Virus was precipitated by adding 5% polyethylene glycol (PEG, mol. wt. 6000) and 2% sodium chloride to supernatant and the pellet was collected after one hour by slow speed centrifugation, suspended in the same buffer overnight at 4°C and clarified by centrifugation at 10K for 10 minutes. Supernatant was given three cycles of PEG precipitation and centrifugation which yielded substantial quantity of virus preparation which showed no reaction against antihost serum in gel immunodifuusion tests.

Antiserum Production and Serological tests: A rabbit was immunized by three intraperitoneal injections of virus (1mg/ml) each at 10-day intervals. The antigen was emulsified with equal volume Freund's complete adjuvant. The rabbit was bled by puncturing the ear vein 10 days after the last injection and a booster injection was administered one month later. Blood samples were collected in beaker and allowed to coagulate overnight at 4°C, serum was separated by centrifugation at 1500 rpm for 10 minutes and preserved at 4°C by adding 0.1% sodium azide.

Titre and purity of antiserum was determined in tube precipitation and two dimensional immunodiffusion tests (Outcherlony, 1960). Tubes containing antigen and antiserum were incubated at room temperature (27°C) for 4 hours, and the agar plates with wells charged with reactants for 24 hours in a moist chamber. Antibody-antigen reactions were noted. In each case the virus was also detected and confirmed by DAS-ELISA (Clark and Adams., 1977). The test antigen consisted of fresh tissue, local lesions from the infectivity hosts, purified virus and debris of tobacco from the field.

Spectrophotometry: Ultraviolet absorption spectrum and concentration of virus was measured in a spectrophometer using quartz cuvettes of 1 cm path length, in the Chemistry Department at the Sultan Qaboos University.

Electron Microscopy: A drop of virus was placed on formvar-coated copper grid and negatively stained with 2% phosphotungstic acid (PTA), pH 6.8. The excess fluid was drained off by means of a piece of filter paper (Hitchborn and Hills, 1965). The grid and the virus preparation were sent to EM laboratory at NARC Islamabad for examination in a Joel Electron Microscope EM 100 CX-II. At least 200 virus particles were measured on an electron micrograph and modal length of the virus particles was calculated.

RESULTS

Symptomatology and Host Range: Tobacco plants under field conditions manifested predominantly mosaic symptoms which were best expressed in young leaves, particularly after topping of infected plants. Other symptoms associated were: mottling in various degrees with dark and green areas, chlorosis, curling, distortion and blistering of leaves, and stunting of entire plant. In some cases, chlorotic and necrotic areas were also found. Infected plants approached early maturity.

The reaction of host species after mechanical inoculation with infective sap is summarized in Table 1. Solanaceous hosts particularly tobacco, tomato, chili, and eggplant were readily infected. Typical symptoms of the virus were reproduced on tobacco, as vein clearing followed by mosaic and mottle. *Nicotiana* spp. in general and *N. glutinosa* in particular developed necrotic local lesions on the inoculated leaves and systemic infection when the plants were held at high temperature (30-32°C). Local infection was observed in datura, *Chenopodium* spp. *Gomphrena*, French bean and cowpea. *N. tabacum* appeared as the best host for the propagation of the virus whereas *C. amaranticolor* as best local lesion assay host (Table 1).

Following plant species were not infected and no virus was recovered or detected in infectivity test and ELISA: *Arachis hypogaea* L. (peanut), *Abelmoschus esculentus* (L.) Moench. (Okra), *Brassica* sp. *Cajanus cajan* (L) Millsp, (pigeon pea), *Carthamus tintorious* L. (safflower), *Cicer arietinum* L. (chick pea), *Canavalia enformis* (L.) DG (sword bean), *Catharanthus roseus* (L.) G. Don. (periwinkle), *Citrullus lanatus* (Thunb) Mansf. (watermelon), *Cucumis melo* L. (muskmelon), *Cyamopsis tetragonoloba* (L.) Taub. (guar), *Crotolaria juncea* L. (sunnhemp), *Glycine max* (L.) Merr. (soybean), *Gossypium hirsutum* L. (cotton), *Helianthus annuus* L. (sunflower), *Lactuca sativa* L. (lettuce), *Pennisetum* sp. (millet), *Petunia hybrida* L. (petunia), *Phaseolus lunatus* L. (Lima bean), *Pisum sativum* L. (garden pea), *Solanum tuberosum* L. (potato), *Sorghum bicolor* L. (sorghum), *Vicia faba* L. (broad bean), *Vigna radiata* (L.) Wilczek, (mashbean), *V. mungo* (L.) Hepper (mungbean), and *Zea mays* L. (corn).

Table 1. Reaction of some host species following mechanical inoculation with tobacco mosaic virus in Oman.

Host species	Common Name	Reaction	
		Local	Systemic
CHENOPODIACEAE			
Chenopodium album L	Chenopodium	LL	--
Ch. amaranticolor C.& R.	Chenopodium	NLL	--
Ch. murale L.	Chenopodium	NLL	--
Ch. quinoa L	Chenopodium	CLL	--
Spinacea oleracea L.	Spinach	CLL	Mo
Beta vulgaris L.	Beet root	L	--
SOLANACEAE			
Capsicum annuun L.	Pepper	L	Mo, LC
C. frutescens L.	Pepper	L	Mo
Datura metel L	Black datura	NLL	Death
D. stramonium L	Jimson weed	NLL	Death
Lycopersicon lycopersicum (L.) Kst.	Tomato	L	Mo
Nicotiana benthamiana Domin.	Tobacco	L	Mo
N. clevelandii Gray	Tobacco	L	Mo, Mot.
N. glutinosa L.	Tobacco	NLL	Mo, Mot.
N. rustica L.	Tobacco	L	VC, Mo, Mot.
N. tabacum L.	Tobacco	L	VC, Mo, LC
N. tabacum L.	Tobacco	L	VC, Mo, LC,.D., Mal.
N. tabacum L.	Tobacco	L	VC, Mo, LC,
Physalis floridana L	Physalis	L	Mot..
Solanum melongena L.	Egg plant	NLL	Mo, Mot.
S. nigrum L.	Black nightshade	L	--
CUCURBITACEAE			
Cucumis sativus L.	Cucumber	L	--
FABACEAE			
Phaseolus vulgaris L.	French bean	CLL	--
Vigna unguiculata (L.) Walp.	Cowpea	LL	--
AMARANTHACEAE			
Gomphrena globosa L.	Button flower	LL	--

Abbreviations: Ch.(chlorosis), CLL (chlorotic), D (distortion), L (latent), LC (leaf curl), LL (local lesions), Mo.(mosaic), Mot.(mottling), N.(necrotic), Mal.(malformation), St.(stunting), VC (vein clearing). Bos, 1970

Physical Properties of Infective Sap: The virus isolate was sap transmissible under wide range of conditions. Aliquots were prepared from standard extracts (1g infected tissue/1ml) in 0.02 phosphate buffer, pH 7.4) and subject to different treatments. Infectivity was assayed on half leaves of *C. amaranticolor.* In 10-fold dilution series, virus was infectious at $1x10^{-7}$. Aliquots were subject to temperatures between 60-90°C at 5°C intervals; virus was completely inactivated at 90°C. Crude sap in screw-capped tubes stored in the laboratory (25-27°C) remained infectious for 30 days, and infected tissue at -4°C for more than one year. The virus was resistant to buffers (borate, phosphate, and citrate) to antioxidants (nicotine sulphate, thioglycollic acid, DIECA) and emulsifying agents (chloroform, carbon tetrachloride and butanol) which were tested and added to the extracts during inoculation and purification procedures.

Properties of purified virus preparation: Purification method involving three cycles of PEG precipitation and centrifugation appeared to be convenient and routinely applicable to consistently obtain substantial yields of virus (12-15 mg/100g tissue). The preparations were infectious, opalescent, and contained numerous unaggregated and unfragmented rigid rods with a modal length of 300 nm. UV absorption spectrum of virus preparations was typical of nucleoprotein, i.e. minimum at 242 nm and maximum at 260, with 260:280 ratios of 1.19 indicating low nucleic acid contents. Virus preparations did not react against antihost serum indicating that they were free from host impurities.

Serological properties: Antiserum prepared against the virus was free from host antibodies as no reaction was observed against host antigen in gel immunodiffusion tests. The antiserum gave a homologous titre of 1:2048 in tube precipitation and 1:512 in agar plates. One strong precipitation and confluent line was observed when the virus was titrated against homologous and imported antiserum of TMV. The virus preparations, inoculated hosts, ratooned tobacco plants and over-summering debris from tobacco fields were ELISA positive.

DISCUSSION

Sultanate of Oman is developing its agriculture on modern and scientific lines leading to sustainability, self sufficiency and higher productivity in majority of the crops. For this purpose, the government is providing to the farmers basic facilities of seed, fertilizer and plant protection material etc. It implies that all the aspects of crop production and protection are unequivocally covered, and surveys and monitoring of pests and diseases and their control constitute an integral part of the programme. We initiated identification of viral diseases prevalent in Oman and this paper presents occurrence, investigations and some characteristics of tobacco mosaic virus.

Tobacco is grown in Oman on a limited scale mainly as a cash crop at Shinas and rarely at other places. Tobacco fields at Shinas were found to be heavily infected with viruses particularly tobacco mosaic virus (TMV) in varying degrees. TMV was identified on the basis of reaction in differential hosts, serology and ELISA, and electron microscopy. Its host range was limited to 23 species across 5 families. The virus was highly infectious, immunogenic and stable. It survived in plant debris in soil after over-summering and had a high thermal inactivation point which was attributed to long summer durations with high temperatures in the country.

TMV is reported to consist of several strains which differ in biological, serological and physical properties and some isolates in particle morphology, and amino acid composition (Hennig and Wittmann, 1972). Regenmortel (1982) listed and distinguished TMV strain groups, based on host infection. TMV isolate under study was identified as common or ordinary strain and its characteristics observed agreed very well with those reported by Zatlin and Israel 1972, Sarkar 1986, Mughal et al. 1986, Hameed et al. 1989). It was distinguished from tomato mosaic virus because it did not infect *N. sylvestris*, and from legume strain because of its lack of infectivity on many legume hosts. The virus isolate reacted strongly in ELISA tests and antiserum to common strain as well with homologous antiserum producing one strong confluent line in gel inmmunidiffusion test. TMV is an important virus disease and because of sparse flora in Oman, is likely to spread to Solanaceous hosts in localized areas due to contaminated seed and movement of plant debris and infected plant material. Therefore, proper phytosanitary measures need to be adopted to check its infections.

ACKNOWLEDGEMENTS

We are highly grateful to Dr. Shaheena Ghazanfer, Associate Professor, Department of Botany, Sultan Qaboos University, for her kind help in the identification of some host plants. Thanks are also due to Dr. Saif Khalid, NARC Islamabad, Pakistan for providing seeds of indicator plants, antiserum and antibodies to common strain of TMV and for electron microscopic examination of virus preparations.

REFERENCES

Agrios, G. 2004. Plant Pathlogy. 4th ed. Elsevier Academic Pr. N.Y.

Bock, K.R. 1972. Identification and partial characterization of plant viruses in the tropic. Trop. Pest Management 28: 399-411.

Bos, L. 1970. Symptoms of Virus Diseases in Plants. Inst. Phytopath. Res. Wageningen, the NL. pp. 206.

Bos, L. 1999. Plant Viruses: Unique and Intriguing Pathogens. Bachuys Publ. Leiden NL. 357 pp.

Clark, M.F. and A.W. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses in plant extracts. Jour. Gen. Virol. 34: 475-483.

Fraenkel-Conrat, H. 1986. Tobacco Mosaic Virus. The history of tobacco mosaic virus and the evolution of molecular biology. In. "The Plant Viruses" 2nd ed.(ed. M.H.V. Regenmortel and H. Fraenkel-Conrat). Plenum press. N.Y.

Hassan, M.M., M.A. Farag and M. El. Taye. 1985. Survey of plant diseases in Sultanate of Oman during 1976. In, "First Int. Conf. of pests and diseases of vegetables and field crops." Egypt. pp. 562- 572.

Hameed, S., S.M. Mughal and B.A. Khan. 1989. Screening of tobacco cultivars for resistance to ordinary strain of tobacco mosaic virus. Pak. Tobacco. 12. 13-16.

Hennig, B. and H.G. Wittmann. 1972. Tobacco mosaic virus: Mutants and Strains. In. "Principles and Techniques in Plant Virology" (Eds. C. Kado and H. Agrawal) van Nostrand Reinhold Pr. pp 546-594.

Hitchborn, J.H and G.H. Hills. 1965. The use of negative staining in electron microscopic examination of plant viruses in crude extracts. Virology. 27: 528-540.

Mughal, S.M., S. Khalid and Z. Hussain. 1986. Isolation, identification and prevalence of tobacco mosaic virus in Pakistan. Pak. Tobacco. 10: 5-9.

Noordam, D. 1973. Identification of Plant Viruses: Methods and Experiments. Centre for Research and Documentation. Wageningen. pp. 50-56.

Outcherlony, G. 1962. Diffusion-in-gel method for immunological analysis. Prog. Allergy 6: 30-154.

Regenmortel, M.H.V. 1982. Serology and Immuno-chemistry of Plant Viruses. Academic Pr. London. pp. 302.

Roger, H. 2002. Matthew's Plant Virology. 4th ed. Academic Pr. (Elsevier imprint) N. Delhi. pp. 1001.

Sarkar, S. 1986. Tobacco Mosaic Virus: Mutants and Strains. In, "The Plant Viruses" vol. 2. (Eds. M.H.Regenmortel and H. Fraenkel-Conrat). Plenum Pr. N.Y. pp. 59-77.

Waller, J.M and J. Bridge. 1978. Plant Diseases and Nematodes in the Sultanate of Oman. PANS 24 (3): 313-326.

Zaitlin, M. and H.W. Israel. 1975. Tobacco Mosaic Virus: Type Strain. Description of Plant Viruses No. 151. CMI/AAB. London.

Zidgali, T.M. and S.M. Mughal. 1993. Status of Pests and Diseases in Oman. 1. Plant Diseases in Batinah. Dir. Agric. Res. Rumais, pp. 150.