A Moroccan Radish Mosaic Virus Isolate from Turnip

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ABSTRACT

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Radish mosaic virus (RaMV) caused a severe disease of turnip (*Brassica rapa* var. *rapa*) in Morocco. Host range and serologic properties of the virus suggested that it was closely related but not identical to strain HZ of RaMV from Yugoslavia. Both viruses were very susceptible to a partial degradation of their light coat proteins. The Moroccan isolate differed markedly from the type strain of RaMV from California. We therefore concluded that the Moroccan isolate belongs to the European subtype of RaMV. This appears to be the first report of RaMV from North Africa.

Turnips are widely grown in Morocco for local consumption in small areas. They have been virtually virus-free, although occasional infections with turnip mosaic virus have been noted (B. E. Lockhart and H. U. Fischer, *unpublished*). In the spring of 1978, a field of turnips with severe viruslike symptoms of an unusual type was found at an experiment station near Marrakesh. Affected plants were dwarfed, rosetted, and slightly chlorotic. Leaves often showed necrotic vein streaking and general necrosis. We present evidence for the identification of the causal agent as radish mosaic virus (RaMV) belonging to the European subtype.

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MATERIALS AND METHODS

Electron microscopy. Crude sap preparations from infected turnip leaves or purified virus preparations were negatively stained with 2% neutralized phosphotungstic acid and viewed under an electron microscope.

Purification, sedimentation analysis, and protein molecular weights. The Moroccan isolate and the European RaMV isolate HZ (17) were purified from systemically infected turnip leaves with either chloroform and butanol (16) or butanol alone (7). Sedimentation velocities (S_{rel}) of virus particles relative to those of top (53S) and bottom (113S) components of belladonna mottle virus (13) and tobacco mosaic virus (194S) (18) were estimated by centrifugation on 10–40% linear sucrose density gradients for 90 min at 35,000 rpm in a Beckman SW 39 rotor. The gradients were fractionated on an Isco fractionator.

Protein molecular weights were determined by sodium dodecyl sulfate polyacrylamide electrophoresis in 7.5% gels as described previously (10).

Serology. Rabbits were immunized by two intramuscular injections 1 wk apart of virus adjusted to a titer of 1:512 and emulsified in Freund's complete or incomplete adjuvant for the first and second injections, respectively. Agar gel double diffusion tests were done with 0.85% Difco Noble agar containing 0.85% sodium chloride, 0.25% sodium azide, and 0.01 M tris-HCl buffer, pH 8.0.

Host range and aphid transmission. Test plants were kept in an insect-proof, air-conditioned greenhouse at 22–27 C.

Inoculum for mechanical transmissions was obtained by grinding affected turnip leaves in 0.05 M phosphate buffer, pH 7.2. Test plants that did not develop symptoms were tested for latent infections by back-inoculation to turnip.

Nonviruliferous, apterous adults of *Myzus persicae*, raised on Tendergreen mustard, and *Aphis fabae*, raised on broad bean, were used for aphid transmission tests. Two tests were carried out with acquisition times of 15 min and 24 hr, respectively, 10 aphids per assay plant, and five plants per test.

RESULTS

Electron microscopy and sedimentation. Crude sap and purified preparations contained numerous isometric particles about 30 nm in diameter, most of which were not penetrated by the stain. In sucrose density gradient centrifugation, the virus yielded three components sedimenting at 57, 103, and 128S.

Serology. The Moroccan isolate reacted with antisera to the American type strain of RaMV (3), the European reference isolate HZ (17), and broad bean stain virus. No reactions were observed with antisera to the isometric viruses turnip yellow mosaic, turnip crinkle, and turnip rosette reported from turnip. Large spurs were observed when the Moroccan isolate was compared with broad bean stain virus or the American type strain of RaMV using antisera to any of these three viruses. However, only a small spur was observed when the virus was compared with the European isolate HZ using the antiserum to the Moroccan isolate (titer 1:2,000) (Fig. 1). With an antiserum to the European isolate HZ (titer 1:2,000), complete fusion of the precipitin lines formed by the two viruses was observed. Nevertheless, intragel cross-absorption tests indicated that this antiserum also had a few antibodies that reacted with the homologous virus but not with the Moroccan isolate. Antisera to the Moroccan isolate and to HZ reacted up to their homologous titers with the American type strain of RaMV, in spite of the large spurs observed with this isolate (Fig. 1).

In immunoelectrophoresis at pH 7.0, the Moroccan isolate and the European and American strains of RaMV migrated toward the anode.

Protein molecular weights. A preparation of the American type strain of RaMV kindly provided by R. N. Campbell yielded two protein bands in sodium dodecyl sulfate polyacrylamide electrophoresis for which molecular weights (mol wt) of 40,000 and 22,000 were calculated. With preparations of the Moroccan isolate (Fig. 2) and the European strain HZ, we observed as many as five bands corresponding to molecular weights of 40,000, 35,000, 24,000, 21,000, and 17,000. Preparations from very young leaves yielded only the 40,000- and the 24,000-mol-wt proteins (Fig. 2, column b). With preparations from older leaves, several different patterns were observed that were not predictable in all details. An increase in the 21,000-mol-wt protein at the expense of the 24,000-mol-wt protein was observed 1) in virus preparations obtained from old leaves compared with preparations obtained from middle-aged leaves of the same plants (Fig. 2, columns c and d); 2) when, during the purification procedure, the expressed plant sap was stored at 4 C for 18 hr before being clarified by chloroform and butanol (Fig. 2, columns e and f); 3) in some cases, when purified preparations were stored at −20 C in 20% glycerol and

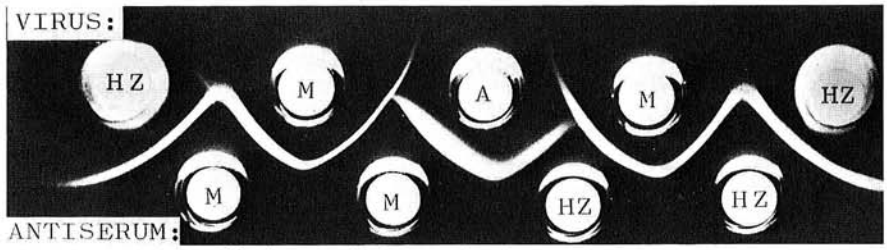

Fig. 1. Agar gel double diffusion test with isolates of radish mosaic virus. Large spurs were formed between the American type strain (A) and the Moroccan isolate (M). A small spur was formed between M and the European strain HZ with an antiserum to M, and no spur was formed with antiserum to HZ.

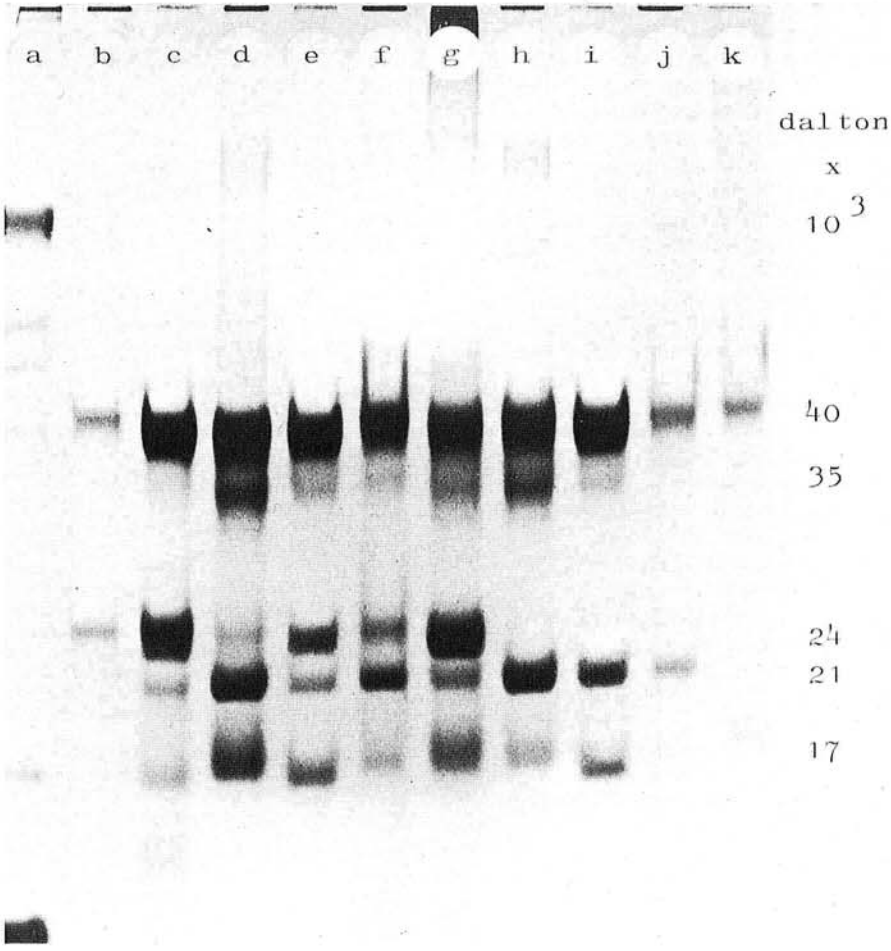

Fig. 2. Sodium dodecyl sulfate polyacrylamide electrophoresis of proteins from preparations of the Moroccan isolate of radish mosaic virus. Column a contains the marker proteins (from top to bottom) bovine serum albumin (67,000 mol wt), fumarase (49,000), egg albumin (43,000), alcohol dehydrogenase (37,000), tobacco mosaic virus coat protein (17,800), and lysozyme (14,300). Preparations for columns b–d were from young (b), middle-aged (c), and old (d) leaves of the same turnip plants. Preparations for columns e–k were from mixtures of middle-aged and old turnip leaves. Preparations for columns e and f were from aliquots of the same crude sap that was treated with chloroform and butanol either immediately (e) or after standing at 4 C overnight (f). Columns g and h contain the proteins from a virus preparation that was either stored 5 days at −20 C and thawed only once just before splitting (g) or was stored 42 days at −20 C and thawed and refrozen repeatedly during this time (h). Column i contains the proteins of the same virus preparation as in column e but treated for 90 min at 37 C with trypsin at a final concentration of 0.01% before splitting. Columns j and k are examples of protein patterns observed with further preparations.

were frozen and thawed repeatedly (Fig. 2, columns g and h); and 4) when purified preparations were treated with 0.01% trypsin (Fig. 2, columns e and i) or chymotrypsin for 90 min at 37 C. The 21,000-mol-wt protein seemed to be a degradation product of the 24,000-mol-wt protein. In some preparations, the 24,000-mol-wt protein had disappeared completely (Fig. 2, columns h–k). A few preparations from old leaves contained neither the 24,000- nor the 21,000-mol-wt protein (Fig. 2, column k). Treating purified virus preparations with trypsin or chymotrypsin did not influence the strength of the 35,000- and 17,000-mol-wt bands. Therefore, whether these bands represent virus proteins or contaminating host proteins is uncertain. No serologic differences were detected among virus preparations yielding different protein patterns.

Host range. Simultaneous host range studies involving 57 plant species from 11 families were done with the Moroccan isolate, the American type strain of RaMV, and the European reference strain HZ. The Moroccan isolate differed only slightly from HZ in that it occasionally caused systemic infection in radish and *Chenopodium murale* and induced local lesions in *Nicotiana clevelandii*. Neither isolate infected cabbage cultivars. In one case, latent systemic infection of broccoli was observed with the Moroccan isolate.

The American type strain differed from the other two isolates by readily infecting cabbage, radish, and collard and by causing local lesions in *N. tabacum*, *N. glutinosa*, and *Gomphrena globosa*. Its effect on turnip was considerably more severe than that of the other two isolates in inducing necrotic local lesions and severe systemic necrosis, which frequently killed the plant.

Stability in sap. In three experiments, the thermal inactivation point of the Moroccan isolate was between 80 and 85 C, the dilution end point was between 10^{-6} and 10^{-7}, and longevity in vitro was between 16 and 32 days at room temperature.

Aphid transmission. Attempts to transmit the virus by *Myzus persicae* or *Aphis fabae* from turnip to turnip or from Tendergreen mustard to Tendergreen mustard in either the persistent or the nonpersistent manner were unsuccessful.

DISCUSSION

The Moroccan turnip virus isolate closely resembles isolates of the European subtype of RaMV that have been isolated from several cruciferous plants but rarely infect radish or cabbage cultivars (1,9,11,14,15,17). In spite of differences in host range, these isolates are barely (9) or not at all (14) distinguishable serologically. This corresponds with our observation of a small spur between the Moroccan isolate and the European isolate HZ with an antiserum to the former but not to the latter. The European and Moroccan isolates are clearly differentiated from the American (3) and Japanese (4) isolates that infect radish and cabbage and, in spite of very similar serum titers, form large spurs when compared with the isolates of the European subtype in the agar gel double diffusion test (14 and this paper).

The Moroccan isolate and the European strain HZ resemble some other comoviruses (2,6,8,12), though not all (5; R. Koenig, *unpublished*), in that their light proteins may become partially degraded in vivo and in vitro to yield a smaller protein with a molecular weight of 21,000. The nature of two other proteins in many of our preparations from older leaves is uncertain.

This is the first report of RaMV in Morocco. The virus appears to occur rather sporadically; however, its destructiveness in turnip, frequency in several parts of Europe (1,9,11,14,15,17), and ability to persist in a number of cruciferous weeds make it a potential threat to the turnip crop in Morocco.

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LITERATURE CITED

1. Avgelis, A., and Quacquarelli, A. 1973. Virus diseases of vegetable crops in Apulia. XII. A mosaic of *Sinapsis nigra* L. Phytopathol. Mediterr. 12:48-53.
2. Blevings, S., and Stace-Smith, R. 1976. In vivo and in vitro effects on the electrophoretic forms of particles of broad bean true mosaic virus. J. Gen. Virol. 31:199-210.
3. Campbell, R. N. 1964. Radish mosaic virus, a crucifer virus serologically related to strains of bean pod mottle virus and to squash mosaic virus. Phytopathology 54:1418-1424.
4. Campbell, R. N., and Tochihara, H. 1969. Radish enation mosaic virus is a strain of radish mosaic virus. Phytopathology 59:1756-1757.
5. Fribourg, C. E., Jones, R. A. C., and Koenig, R. 1977. Andean potato mottle, a new member of the cowpea mosaic virus group. Phytopathology 67:969-974.
6. Geelen, J. L. M. C., van Kammen, A., and Verduin, B. J. M. 1972. Structure of the capsid protein of cowpea mosaic virus. The chemical subunit: Molecular weight and number of subunits per particle. Virology 49:205-213.
7. Hollings, M., and Stone, O. M. 1969. Purification and serological reactions of four polyhedral viruses from Cruciferae. Zentralbl. Bakteriol. Parasitenkd. Abt. 2 132:237-248.
8. Jones, A. T., and Barker, H. 1976. Properties and relationships of broad bean stain virus and echtes Ackerbohnenmosaik-Virus. Ann. Appl. Biol. 83:231-238.
9. Kassanis, B., White, R. F., and Woods, R. D. 1973. Genetic complementation between middle and bottom components of two strains of radish mosaic virus. J. Gen. Virol. 20:277-285.
10. Koenig, R., Stegemann, H., Francksen, H., and Paul, H. L. 1970. Protein subunits in the potato virus X group. Determination of the molecular weights by polyacrylamide gel electrophoresis. Biochim. Biophys. Acta 207:184-189.
11. Mamula, D., Miličić, D., Štefanac, Z., and Horvath, J. 1972. Neue Angaben über Verbreitung und Wirtspflanzen des Rettichmosaik-Virus (radish mosaic virus). Acta Phytopathol. Acad. Sci. Hung. 7:369-375.
12. Niblett, C. L., and Semancik, J. S. 1969. Conversion of the electrophoretic forms of cowpea mosaic virus in vivo and in vitro. Virology 38:685-693.
13. Paul, H. L. 1971. Belladonna mottle virus. Descriptions of Plant Viruses, No. 52. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
14. Plakolli, M., and Štefanac, Z. 1976. Serological and other relationships among the isolates of radish mosaic virus. Phytopathol. Z. 87:114-119.
15. Shukla, D. D., and Schmelzer, K. 1974. Studies on viruses and virus diseases of crucifer plants. XVI. Purification and serology of a GDR isolate of radish mosaic virus and its comparison with an American and a Yugoslavian isolate. Acta Phytopathol. Acad. Sci. Hung. 9:227-235.
16. Steere, R. L. 1956. Purification and properties of tobacco ringspot virus. Phytopathology 46:60-69.
17. Štefanac, Z., and Mamula, D. 1971. A strain of radish mosaic virus occurring in turnip in Yugoslavia. Ann. Appl. Biol. 69:229-234.
18. Zaitlin, M., and Israel, H. W. 1975. Tobacco mosaic virus (type strain). Descriptions of Plant Viruses, No. 151. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 6 pp.