Serological Identification of Four Tobamoviruses Infecting Pepper

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ABSTRACT

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Four tobamoviruses infecting cultivars of pepper were identified by immunodiffusion tests and host plant reactions. The white mosaic virus strain (ATCC PV 230) was identified as a strain of type tobacco mosaic virus (TMV). It was very similar in host reactions to the yellow strain of TMV from *Nicotiana glauca* (ATCC PV 223), which was also shown to be a strain of type TMV. The South Carolina mottling strain of TMV (ATCC PV 228) was identified as a strain of para-tobacco mosaic virus (PTMV ≈ strain U2 ≈ G-TAMV). McKinney's latent strain of TMV from South Carolina (ATCC PV 227) was found to be very similar if not identical to the newly described pepper mild mottle virus (PMMV) from Sicily. Another virus sample from Italy contained PMMV and tomato mosaic virus (ToMV).

Wild strains of tobacco mosaic virus (TMV) that infect species and cultivars of the genus *Capsicum* are of great economic importance because they may be the most deleterious viruses in this crop (3,5–7,12,23). A detailed survey on diseases of pepper caused by strains of TMV has been published (24), but these strains could not be identified serologically at that time. Recently, a tobamovirus named pepper mild mottle virus (PMMV) was isolated from TMV-resistant pepper in Sicily (32). Besides this virus, three other tobamoviruses are able to incite diseases in pepper crops. In addition to type TMV, tomato mosaic virus (ToMV) was found to infect peppers in the United States (22) and in Italy (6). More recently, para-TMV (PTMV ≈ strain U2), which is widespread in field tobacco in Germany (30), was detected in pepper crops in Piedmont and Umbria in Italy (5, C. Wetter, *unpublished*). This research was expanded to study some tobamoviruses causing infections in pepper of the collection of the late H. H. McKinney. The viruses were kindly supplied in 1978 by R. W. Fulton and were not investigated earlier because there was no indication that viruses inducing similar diseases in pepper were present in Europe. After it was found that at least four tobamoviruses occurred here (32), attempts were made to identify some of McKinney's strains along with a new virus sample obtained from pepper in Italy.

MATERIALS AND METHODS

Virus sources. The tobamoviruses used for comparative studies were from stock laboratory cultures as described previously (32). Four pepper virus strains investigated are listed in the catalogue of the American Type Culture Collection (ATCC) under plant viruses (PV) (2). Further details on these strains are presented under Results. In addition to the ATCC strains, a virus sample from a pepper fruit with severe mosaic symptoms was studied. It was kindly supplied by M. Marte, Perugia, Italy.

Host range and symptomatology. Dried leaf material of the ATCC strains and of the virus from Italy were soaked for about 1 hr in a few milliliters of 0.01 M phosphate buffer, pH 7.0, and ground. The sap was inoculated to celite-dusted leaves of the following plants: *Chenopodium quinoa* Willd. (Chenopodiaceae); *Capsicum annuum* L. 'Sperling's Merit,' 'Mariza,' 'Goldtopas,' and 'Yolo Wonder'; *C. frutescens* L.; *Datura stramonium* L.; *Lycopersicon esculentum* Mill. 'Haubners Vollendung' and 'Sweet 100'; *Nicotiana debneyi* Domin.; *N. clevelandii* Gray; *N. glutinosa* L.; *N. sylvestris* Speg. & Comes; and *N. tabacum* L. 'Samsun' and 'Xanthi-nc' (Solanaceae).

Purification. Virus was purified from plants inoculated with the original inoculum by the polyethylene-glycol-precipitation method (11) followed by several cycles of differential centrifugation. Virus concentrations were determined by using a specific extinction coefficient of 3.1, which roughly agrees with the values for TMV, PTMV, and PMMV (32).

Immunodiffusion tests. Agar gel immunodiffusion tests were conducted on slides as described previously (32). The optimal proportions for reactions were determined in preliminary tests with dilution rows of antigen and antibody. The first tests were made with purified antigen (about 10 mg/ml) obtained from plants inoculated with the original inoculum. A second series of serological tests was carried out with virus transferred to a second set of host plants. Tests were repeated a third time with virus obtained from plants once again inoculated with the original inoculum. Immunodiffusion tests were confirmed by intragel-absorption tests (27).

RESULTS

The different isolates from pepper could be identified in immunodiffusion tests with either TMV, ToMV, PTMV, or PMMV. These four viruses are easily distinguished by a strong spur formation (Fig. 1A). Every possible combination of homologous and heterologous reactions with the four antisera were tested, but in the characterization of the unknown isolates, mostly tests that show a fusion of precipitin lines in the homologous reactions are shown.

No reliable method was found to ensure that local lesions were formed in local lesion hosts when pepper was the source of virus inoculum. All peppers contain a strong inhibitor of virus infection (9,17) that prevents the formation of local lesions even at high virus concentrations, especially in tobaccos such as *N. sylvestris, N.*

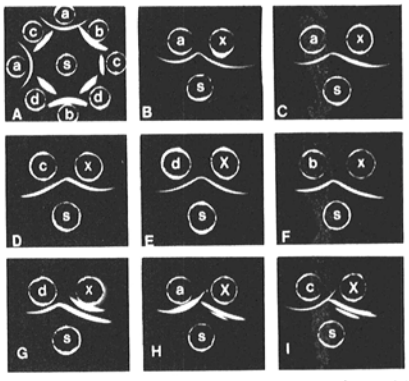

Fig. 1. Immunodiffusion tests with antisera (s) to tobacco mosaic virus (TMV), para-tobacco mosaic virus (PTMV), pepper mild mottle virus (PMMV), and tomato mosaic virus (ToMV), and with homologous and heterologous antigens. **(A)** Central well (s) contained TMV antiserum; peripheral wells contained: a = TMV, b = ToMV, c = PTMV, d = PMMV. **(B)** s = TMV antiserum, a = TMV, and x = PV 230. **(C)** s = TMV antiserum, a = TMV, and x = PV 223. **(D)** s = PTMV antiserum, c = PTMV, and x = PV 228. **(E)** s = PMMV antiserum, d = PMMV, and x = PV 227. **(F)** s = ToMV antiserum, b = ToMV, and x = antigen from the Italian sample. **(G)** s = PMMV antiserum, d = PMMV, and x = antigen from the Italian sample. **(H)** s = TMV antiserum, a = TMV, and x = antigen from the Italian sample. **(I)** s = PTMV antiserum, c = PTMV, and x = antigen from the Italian sample.

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glutinosa, and Xanthi. The inhibitor acts less on *Datura stramonium*, the most sensitive local lesion host (17). To obtain reliable local lesion reactions, the virus from pepper was transferred to Samsun tobacco, *N. debneyi* or *N. clevelandii*, and after multiplication, to the local lesion hosts.

White and yellow mosaic strains of TMV. The white mosaic strain was isolated by E. M. Johnson in 1928 from *Capsicum frutescens* 'Grossum' in Kentucky (13). It was propagated by McKinney (2) in Samsun tobacco and is listed as PV 230 (ATCC). In TMV-resistant peppers, the virus caused necrotic local lesions and leaf drop. Only occasionally, the virus caused systemic yellow flecks from which the virus could be recovered. The first symptoms in Samsun tobacco were distinct circular yellow spots on the rubbed leaves (Fig. 2A). These spots increased in diameter by forming a light halo. Systemically infected leaves developed a striking creamy white mosaic (Fig. 2B), which predominated through several transfers in Samsun tobacco. In *N. sylvestris*, the virus caused necrotic local lesions but did not become systemic as the ordinary strains of type TMV do.

Immunodiffusion tests resulted in a fusion of lines when isolate PV 230 was tested next to type TMV against TMV antiserum (Fig. 1B) but showed a strong spur when it was tested next to the homologous antigen against antisera to all other TMV strains. Johnson (13) reported that the white mosaic strain resembled the yellow mosaic strain of TMV isolated by McKinney (18) from *N. glauca* on Gran Canaria. In further studies, this virus strain was called yellow mosaic type B (19) and is listed as PV 223 (ATCC). The symptoms caused by the yellow mosaic strain on different host plants, including peppers, were very similar to those described for the white strain (Fig. 2C). In contrast to the white strain, however, green strains present in the yellow mosaic strain population predominated after several transfers in Samsun tobacco. The bright yellow mosaic developed most prominently at a high greenhouse temperature (>30 C during the day). Inoculated leaves of Samsun tobacco produced circular yellow spots similar to the white mosaic strain. In *N. sylvestris*, the yellow mosaic strain caused local lesions but did not become systemic. Immunodiffusion tests resulted in a fusion of lines when PV 223 was tested next to TMV against TMV antiserum (Fig. 1C) and spur formation when tested next to ToMV, PTMV, and PMMV. The results indicate that the white mosaic strain from pepper and the yellow strain from *N. glauca* are minor variants of type TMV, with remarkable similarities in host reactions.

South Carolina mild mottling strain of TMV. The virus was isolated by McKinney in 1949 from pungent pepper in South Carolina and Georgia (20). It was propagated in Samsun tobacco and dried material of this strain, designated PV 228 (ATCC), was used in the present studies. The original description of host reactions is very similar to that of the pepper virus called "latent strain of TMV" (PV 227) (20). PV 228 could easily be distinguished, however, by immunodiffusion tests. The virus gave strong spurs when tested next to TMV, ToMV, and PMMV against the homologous antisera. A fusion of precipitin lines occurred in tests with PTMV antigen and antiserum (Fig. 1D). Additional tests were carried out with antigens and antisera to the following strains: U2 (26), mild strain of TMV (14), and the green mottling strain of tomato atypical mosaic virus (G-TAMV) (15). In all cases, a fusion of the lines indicated that these different antigens could not be distinguished by this method.

Latent strain of TMV. The latent strain of TMV (LSTMV) was isolated from pungent pepper in South Carolina and first described by McKinney (20). It was also studied by Greenleaf et al (10), and strains of this virus were found in Alabama and Florida. The virus infected all pepper varieties that carry the *L*-gene for TMV resistance (12). The virus was multiplied in Samsun tobacco by McKinney and is listed as PV 227 (ATCC). In the present studies after infection with LSTMV, most cultivars of pepper developed a mild mosaic under greenhouse conditions. In contrast to TMV, ToMV, and PTMV, LSTMV did not cause leaf drop in the TMV-resistant pepper cultivars Yolo Wonder and Sperling's Merit. With some cultivars, it was difficult to decide by visual inspection if a plant was healthy or diseased. Immunodiffusion tests with purified virus showed spur formation with TMV, ToMV, and PTMV antisera but a fusion of lines when tested next to PMMV against PMMV antiserum (Fig. 1E).

In contrast to the host range studies of McKinney (20) and Greenleaf et al (10), who reported that LSTMV infects Samsun tobacco systemically, virus could not be recovered from uninoculated leaves of infected plants. Several trials were made but in no case could a systemic spread of the virus be observed after it had multiplied in the inoculated leaves. In further studies, McKinney (21) stated that LSTMV has little or no affinity for newly formed leaves of Samsun tobacco. In contradiction to his earlier results, he was not able to demonstrate a systemic multiplication. It seems clear from the present results and those of McKinney (21) that Samsun tobacco cannot be infected systemically.

Tomato mosaic virus. Virus purified from pepper infected with the virus sample from Italy gave a fusion of precipitin lines when tested next to ToMV against antiserum to ToMV (Fig. 1F). A similar fusion of lines was observed with respect to PMMV (Fig. 1G). A spur resulted in tests with TMV and PTMV antigens and antisera (Fig. 1H,I). Thus, the pepper from Italy was doubly infected with ToMV and PMMV. Intragel absorption tests confirmed these findings. Host reactions were also in accordance with the serological results. In Xanthi tobacco, *N. sylvestris* and *N. glutinosa*, two kinds of local lesions were found, large ones caused by ToMV and small ones caused by PMMV (Fig. 3). Both virus strains could be separated by specific hosts. Samsun tobacco selected for ToMV, which became systemic, whereas PMMV remained local. In contrast, ToMV-resistant cultivars of pepper were systemically infected by PMMV and not by ToMV.

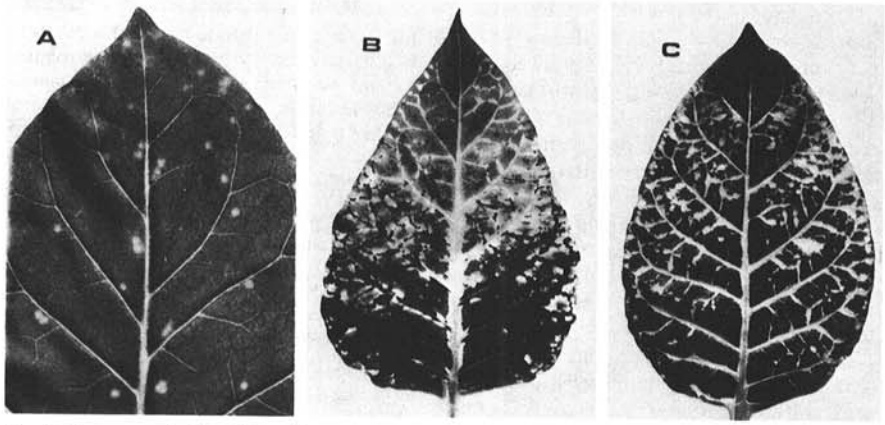

Fig. 2. Symptoms induced by white and yellow mosaic strains of tobacco mosaic virus (TMV) in Samsun tobacco. **(A)** Circular yellow spots in leaves inoculated with PV 230. **(B)** Systemically infected leaf showing white mosaic and white veinbanding induced by PV 230. **(C)** Creamy white veinbanding of a leaf systemically infected with PV 223.

DISCUSSION

Identification of tobamoviruses from peppers by host plant reactions was difficult because of the inhibitor that prevented formation of local lesions

Fig. 3. Leaf of *Nicotiana glutinosa* inoculated with virus from the Italian sample. Two kinds of lesions were formed: small white dots induced by PMMV and fewer and larger necrotic local lesions induced by ToMV.

(9,17). Preliminary experiments to block the inhibitor were unsuccessful. The TMV-resistant pepper cultivars Yolo Wonder and Sperling's Merit reacted with leaf drop to inoculation with TMV, ToMV, and PTMV as an indication of resistance. TMV and ToMV generally did not become systemic, but PTMV caused a systemic infection in addition to the leaf drop. In some cultivars, plants were killed when infected early. PTMV (16), U2 (26), and G-TAMV (15) have a very similar amino acid composition (27,28) and known amino acid sequence (1,25,33). They cannot be distinguished antigenically by immunodiffusion tests and may be regarded as strains of one virus. The virus is probably widespread in Europe because it is common in field tobacco (30) and in European cigarettes (31), from which it may be transferred to pepper. Since a strain of this virus (PV 228) was found to have infected pepper 30 yr ago in South Carolina and Georgia (20) and recently in Italy (5), it may have a worldwide distribution.

PMMV, recently described as a virus that infects all pepper cultivars tested so far (10,32), caused only mild symptoms and no leaf drop in peppers. It is very similar if not identical to LSTMV. The name LSTMV is not appropriate because the virus is latent only in the inoculated leaves but does not become systemic in Samsun tobacco. The proposed name PMMV (32) seems to be more adequate and agrees with the biological properties (10,20). New reports on the virus should therefore refer to PMMV (≈LSTMV), first described by McKinney (20). The electrophoretic mobility of LSTMV was studied by Ball (4), but the virus was found to be contaminated with type TMV. In the present study, a contamination of LSTMV with TMV could not be excluded with certainty because PV 227 was multiplied first in TMV-resistant peppers. According to McKinney (21), traces of TMV possibly could be carried in a culture of LSTMV in Samsun tobacco through several transfers before being expressed, because LSTMV suppresses the multiplication of TMV. Some of our results support these findings.

The pepper cultivar from Italy, infected with PMMV and ToMV, was chosen as an example of double infection. The reaction of pepper cultivars to infection with a tobamovirus from tomato, called tomato atypical mosaic virus (TAMV), was studied by Miller and Thornberry (22). This virus was further characterized by Knight et al (15) and called Y-TAMV. Its amino acid composition was very similar to that of the dahlemense strain of ToMV (29). ToMV was found to infect pepper crops in Italy (6) and France (M. H. V. van Regenmortel, *personal communication*).

In recent serological tests with the TMV strain from pepper described from Argentina (8), it was different from PMMV (32) and the viruses identified in this paper (C. Wetter, *unpublished*). Four or possibly five tobamoviruses with a worldwide distribution have to be considered in attempts to breed for the so-called TMV resistance in pepper. Because some of these viruses are known to be seedborne (7,10,20), breeders should be aware of the danger of introducing virus by the exchange of pepper seed.

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LITERATURE CITED

1. Altschuh, D., Reinbolt, J., and van Regenmortel, M. H. V. 1981. Sequence and antigenic activity of the region 93-113 of the coat protein of strain U2 of tobacco mosaic virus. J. Gen. Virol. 52:363-366.
2. American Type Culture Collection. Catalogue of Strains II. 3rd Ed., Rockville, MD. 1981. 413 pp.
3. Anderson, C. W., and Corbett, M. K. 1957. Virus diseases of peppers in Central Florida. Survey results 1955. Plant Dis. Rep. 41:143-147.
4. Ball, E. M. 1966. A technique for comparing the electrophoretic mobility rates of viruses or virus strains. Arch. Biochem. Biophys. 114:547-556.
5. Conti, M., and Marte, M. 1983. Virus, virosi e micoplasmosi del peperone. Ital. Agric. 120:132-152.
6. Conti, M., and Masenga, V. 1977. Identification and prevalence of pepper viruses in Northwest Italy. Phytopathol. Z. 90:212-222.
7. Demski, J. W. 1981. Tobacco mosaic virus is seedborne in pimiento peppers. Plant Dis. 65:723-724.
8. Feldman, J. M., and Oremianer, S. 1972. An unusual strain of tobacco mosaic virus from pepper. Phytopathol. Z. 75:250-267.
9. Fischer, H., and Nienhaus, F. 1973. Virushemmende Prinzipien in Paprikapflanzen (*Capsicum annuum* L.). Phytopathol. Z. 78:25-41.
10. Greenleaf, W. H., Cook, A. A., and Heyn, A. N. J. 1964. Resistance to tobacco mosaic virus in *Capsicum*, with reference to the Samsun latent strain. Phytopathology 54:1367-1371.
11. Hebert, T. T. 1963. Precipitation of plant viruses by polyethylene glycol. Phytopathology 53:362.
12. Holmes, F. O. 1934. Inheritance of ability to localize tobacco mosaic virus. Phytopathology 24:984-1002.
13. Johnson, E. M. 1930. Virus diseases of tobacco in Kentucky. Pages 285-415 in: Ky. Agric. Exp. Stn. Bull. 306.
14. Johnson, J. 1947. Virus attenuation and the separation of strains by specific hosts. Phytopathology 37:822-837.
15. Knight, C. A., Silva, D. M., Dahl, D., and Tsugita, A. 1962. Two distinctive strains of tobacco mosaic virus. Virology 16:236-243.
16. Köhler, E., and Panjan, M. 1943. Das Paramosaikvirus der Tabakpflanze. Ber. Dtsch. Bot. Ges. 61:175-180.
17. McKeen, C. D. 1956. The inhibitory activity of extract of *Capsicum frutescens* on plant virus infections. Can. J. Bot. 34:891-903.
18. McKinney, H. H. 1929. Mosaic diseases in the Canary Island, West Africa, and Gibraltar. J. Agric. Res. 39:557-578.
19. McKinney, H. H. 1935. Evidence of virus mutation in the common mosaic of tobacco. J. Agric. Res. 51:951-981.
20. McKinney, H. H. 1952. Two strains of tobacco mosaic virus, one of which is seed-borne in an etch-immune pungent pepper. Plant Dis. Rep. 36:184-187.
21. McKinney, H. H. 1968. Further study of the latent strain of the tobacco mosaic virus. Plant Dis. Rep. 52:919-922.
22. Miller, P. M., and Thornberry, H. H. 1958. A new viral disease of tomato and pepper. Phytopathology 48:665-670.
23. Murakishi, H. H. 1960. A necrotic pod streak of pepper caused by tobacco mosaic virus. Phytopathology 50:464-466.
24. Ramakrishnan, K. 1961. Virus diseases of pepper (*Capsicum* spp.). J. Indian. Bot. Soc. 15:12-46.
25. Rentschler, L. 1967. Aminosäuresequenzen und physikochemisches Verhalten des Hüllproteins eines Wildstammes des Tabakmosaikvirus. I. Analyse der Primärstruktur (Pos. 62-134) des Hüllproteins vom Wildstamm U2. Mol. Gen. Genet. 100:84-95.
26. Siegel, A., and Wildman, S. G. 1954. Some natural relationships among strains of tobacco mosaic virus. Phytopathology 44:277-282.
27. Van Regenmortel, M. H. V. 1967. Serological studies on naturally occurring strains and chemically induced mutants of tobacco mosaic virus. Virology 31:467-480.
28. Van Regenmortel, M. H. V. 1981. Tobamoviruses. Pages 541-564 in: Handbook of Plant Virus Infections and Comparative Diagnosis. E. Kurstak, ed. Elsevier/North Holland Biomedical Press, Amsterdam. 943 pp.
29. Wang, A. L., and Knight, C. A. 1967. Analysis of protein components of tomato strains of tobacco mosaic virus. Virology 31:101-106.
30. Wetter, C. 1980. Occurrence of para-tobacco mosaic virus in field tobacco in South-West Germany. Z. Pflanzenkr. Pflanzenschutz 87:150-154.
31. Wetter, C., and Bernard, M. 1977. Identifizierung, Reinigung und serologischer Nachweis von Tabakmosaikvirus und Para-Tabakmosaikvirus aus Zigaretten. Phytopathol. Z. 90:257-267.
32. Wetter, C., Conti, M., Altschuh, D., Tabillion, R., and van Regenmortel, M. H. V. 1984. Pepper mild mottle virus, a tobamovirus infecting pepper cultivars in Sicily. Phytopathology 74:405-410.
33. Wittmann, H. G. 1965. Die primäre Proteinstruktur von Stämmen des Tabakmosaikvirus. Teil IV. Aminosäuresequenzen (Pos. 1-61 und 135-158) des Proteins des Tabakmosaikvirus-Stammes U2. Z. Naturforsch. 20b:1213-1223.