Identification and Comparison of Two Isolates of Tomato Bushy Stunt Virus from Pepper and Tomato in Morocco

H. U. Fischer and B. E. L. Lockhart

Laboratoire de Virologie, Direction de la Recherche Agronomique, Phytiatrie, Rabat, Morocco; and Department of Plant Pathology, University of Minnesota, St. Paul/Laboratoire de Virologie, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco.

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ABSTRACT

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Two variants of tomato bushy stunt virus (TBSV) were isolated from tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*), respectively, in different regions of Morocco. The tomato isolate was serologically identical to the type strain (American Type Culture Collection PV 90) whereas the pepper isolate could be clearly differentiated by test plant and serological reaction. This is apparently the first report of natural infection of pepper by TBSV.

Intensive tomato production in Morocco is concentrated along the Atlantic littoral between Rabat and Safi and south of Agadir. One of the limiting factors in tomato production is the occurrence of virus diseases, the most important of which is caused by tomato mosaic virus (TMV) (11). Cucumber mosaic virus (CMV) and potato virus Y (PVY) also have been isolated repeatedly from tomatoes (Lockhart and Fischer *unpublished*), but these do not appear to be economically important on this crop. In the spring of 1975, conspicuous chlorotic ring and band patterns (Fig. 3) were observed on tomato fruits in several localities. Plants that produced fruits with similar symptoms were found in a tomato-growing area south of Casablanca. These plants were badly stunted and had an abnormal, bushy appearance. The basal leaves were usually normal whereas the top leaves were distorted and filiform. In 1976, the disease was observed again on large numbers of tomato fruits on local markets, and on tomato plants in fields along the Atlantic coast. Because tomato fruits showing virus symptoms are unacceptable for export, such fruit must be disposed of on local markets at substantially lower prices. Along the Atlantic littoral, where tomato production is very largely export-oriented, this disease may therefore be considered to be potentially very important.

In the course of a routine pepper virus disease survey in the Rabat-Salé region, carried out in the spring of 1975, a virus was isolated which behaved differently in test plant reactions from PVY and CMV, the most prevalent pepper viruses in Morocco (10, 12). Subsequent tests revealed both the pepper and tomato virus isolates to be strains of TBSV differing in host reaction and antigenic properties.

MATERIALS AND METHODS

All test plants were raised in steam sterilized soil and kept in an insect-proof, air-conditioned greenhouse. Routine inoculations were made with fresh leaf tissue ground in 0.05 M phosphate buffer, pH 7.2.

Physical properties.—All physical property determinations were made using the same sample of freshly extracted crude sap of inoculated tomato leaves. *Chenopodium quinoa* Willd. served as the assay host. In determining the dilution end point (DEP), distilled water was used as the diluent.

Virus purification.—Virus was purified by the method of Hollings (5), followed by rate-zonal density gradient centrifugation.

Electron microscopy.—Dip preparations and purified virus were stained with 2% neutralized phosphotungstic acid (PTA) prior to electron microscopy.

Serological tests.—Serological experiments were carried out in agar gel double-diffusion plates of 0.5% agarose and 0.02% sodium azide prepared in 0.1 M citrate buffer, pH 6.5. Tomato bushy stunt virus antisera designated respectively AF (against petunia asteroid mosaic virus), BS-3 (against TBSV strain BS-3) and PLCV (against pelargonium leaf curl virus), with homologous titers of 1:128, 1:256, and 1:512, respectively, were provided by the Institut für Virusserologie, Braunschweig, Germany. Tomato bushy stunt virus antiserum ATCC PVAS 81 with a homologous titer of 1:128 was obtained from the American Type Culture Collection. The virus concentration used in immunodiffusion tests was 1 mg/ml. All antisera were diluted 1:3 prior to testing.

RESULTS

Host range and symptoms.—The following plants developed necrotic local lesions following mechanical inoculation with both Moroccan virus isolates: *Cucumis sativus* L. 'National Pickling'; *Cucurbita pepo* L. 'Early Sweet Sugar'; *Nicotiana tabacum* L. 'Samsun', 'White Burley', 'Xanthi-nc'; *N. glutinosa* L.; *N. sylvestris* Spegaz. & Comes; *Petunia hybrida* Vilm.; *Physalis floridana*

Rydb.; *Phaseolus vulgaris* L. 'Bountiful'; *Vicia faba* L.; *Vigna unguiculata* L. (Walp.) 'Early Ramshorn'; *Pisum sativum* L. 'Lincoln'; *Lupinus albus* L.; *Trigonella foenum-graecum* L.; *Antirrhinum majus* L.; *Gomphrena globosa* L.; *Beta vulgaris* L.; *Ocimum basilicum* L.; and *Zinnia elegans* Jacq. No systemic symptoms were observed and no virus could be obtained by back inoculation from noninoculated top leaves to *C. amaranticolor*. Both virus isolates produced local as well as systemic symptoms in *N. clevelandii* Gray and *Solanum melongena* L. 'Black Beauty'.

Differential host response to the pepper and tomato TBSV isolates.—In general, the symptoms elicited by the pepper isolate on test plants were significantly more necrotic and the effect on plant development more pronounced than those produced by the tomato isolate. Several test plants could be used to differentiate the two viruses: all *Chenopodium* spp. tested developed systemic symptoms with the pepper isolate; *C. foetidum* Schrad. developed lethal stem and top necrosis. No systemic invasion of *Chenopodium* species by the tomato isolate occurred. *Datura stramonium* L. was never systemically infected by the pepper isolate, whereas the tomato virus occasionally produced systemic symptoms in this host, especially when very young plants were inoculated. On *D. metel* L. the pepper isolate consistently produced numerous distinct necrotic local lesions followed by abscission of the infected leaves, whereas in the same test plant the tomato isolate produced diffuse chlorotic local lesions, without necrosis or abscission (Fig. 1). Both virus isolates produced necrotic lesions on inoculated leaves of spinach (*Spinacia oleracea* L.), but the pepper isolate, in addition, produced severe stunting and lethal systemic necrosis. The tomato isolate produced no systemic symptoms in spinach, and could not be recovered from the young leaves. *Lycopersicon pimpinellifolium* Mill. was infected locally by both virus isolates, but was systemically infected only by the tomato isolate. The pepper isolate produced local necrotic lesions on *Tropaeolum majus* L., which was immune to infection by the tomato TBSV isolate.

When the two virus isolates were tested on 10 pepper cultivars, the pepper TBSV isolate caused severe symptoms, which were characterized by leaf deformation, stunting, leaf and flower abscission, and lethal systemic necrosis. The tomato TBSV isolate produced only mild symptoms on eight of the cultivars, and no symptoms on the remaining two. The two virus isolates produced similar symptoms on the 10 tomato cultivars tested. Local symptoms consisted of chlorotic and necrotic lesions. Systemic infection occurred infrequently, and caused severe stunting, necrosis and foliar deformation similar to

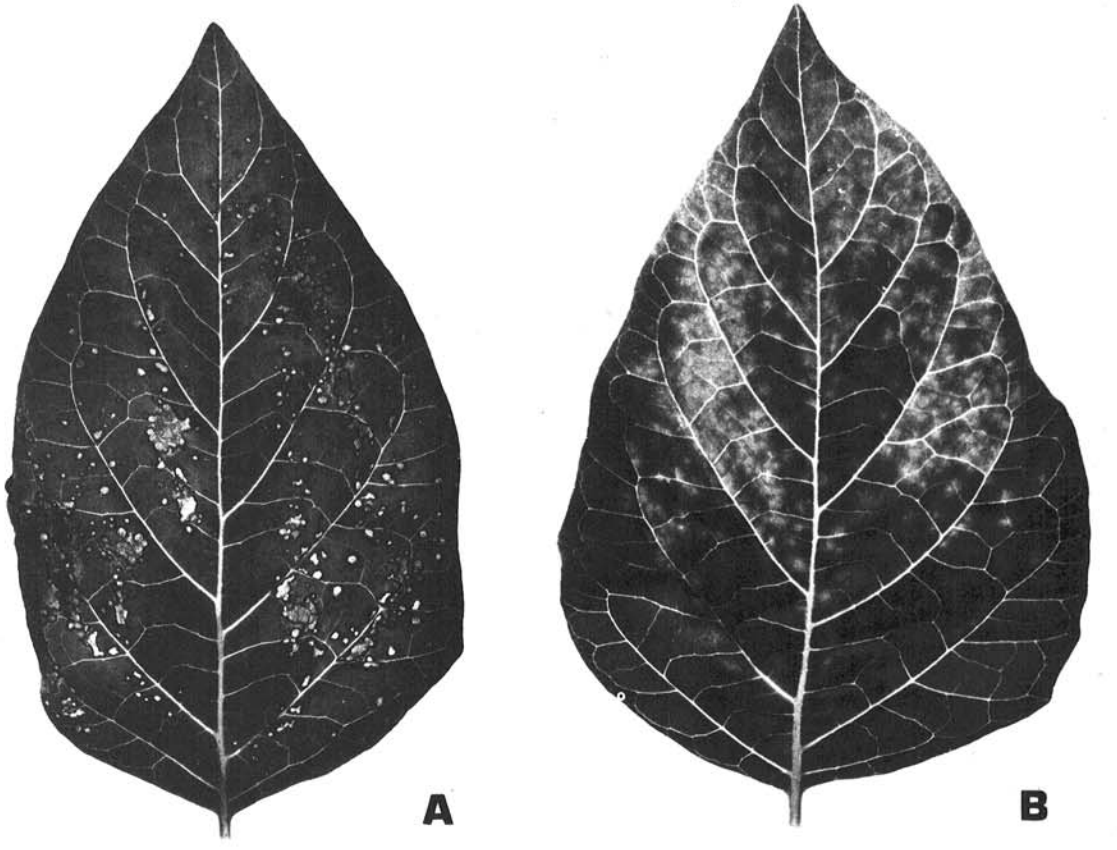

Fig. 1-(A, B). A) Local reaction of *Datura metel* to infection with the Moroccan pepper isolate of tomato bushy stunt virus (TBSV) 6 days after mechanical inoculation. **B)** Local reaction of *D. metel* to infection with the Moroccan tomato isolate of TBSV 6 days after mechanical inoculation.

that observed in the field.

Physical properties.—The pepper TBSV isolate had a thermal inactivation point (TIP) of 80-85 C, longevity in vitro (LIV) of 7-8 wk, and a DEP of 10^{-6}-10^{-7}. The tomato TBSV isolate had a TIP of 85-90 C, a LIV in excess of 8 wk, and a DEP of 10^{-3}-10^{-4}.

Virus purification and electron microscopy.—Both Moroccan TBSV isolates as well as the type strain were satisfactorily purified by the method used (5). Isometric virus particles approximately 30 nm in diameter were observed in crude sap of infected leaves and fruit, in partially purified preparations, and in samples taken from virus bands following density gradient centrifugation. In rate-zonal density gradient centrifugation in 10-40% sucrose gradients all three virus isolates sedimented as two UV-absorbing bands. Nearly all the infectivity was associated with the faster-sedimenting band as determined by local lesion assay on *C. quinoa*.

Serology.—In the immunodiffusion experiments, the virus isolates from pepper and tomato and the type strain of TBSV were tested in adjacent wells against four TBSV-antisera. Specific reactions occurred in all virus-antiserum combinations tested. The precipitin lines of the type strain and the tomato isolate always fused with each other, but spurred or intersected with the line of the pepper isolate (Fig. 2-A). The control preparation from healthy tomato leaves, processed in the same way as the viruses, gave no reaction. When TBSV type-strain antiserum was absorbed with the pepper virus and then tested against all three viruses, no reaction was obtained with the pepper isolate, but distinct lines formed with the tomato isolate and the type strain (Fig. 2-B). When type-strain antiserum was absorbed with the tomato virus in the same way, none of the three isolates reacted. Further cross-absorption tests were carried out with PLCV antiserum. After absorption with the pepper virus this antiserum still reacted faintly with the tomato isolate and the type strain, but not with the pepper isolate (Fig. 2-C). Absorption of PLCV antiserum with type-strain virus still permitted the formation of a strong precipitin line with the pepper virus, whereas the tomato virus and the type strain did not react (Fig. 2-D). The same reaction pattern was obtained when PLCV antiserum was mixed with the tomato virus prior to testing. In this case, however, very faint traces of reaction were still visible with the tomato isolate and the type strain.

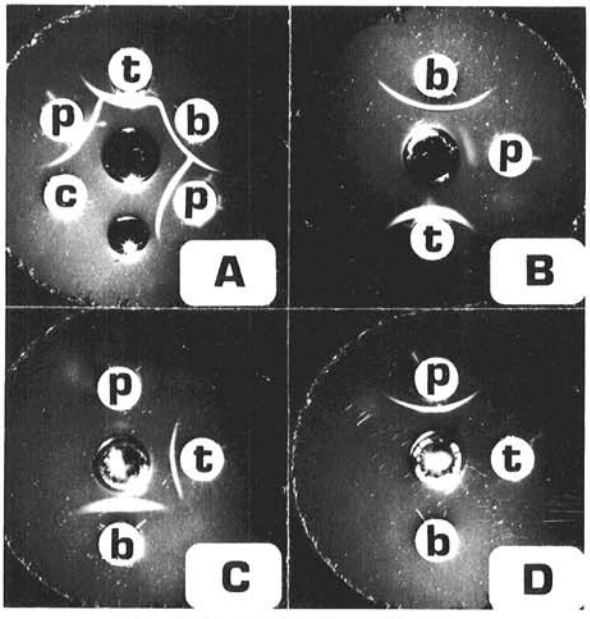

Fig. 2-(A to D). Serological reactions of the Moroccan pepper and tomato bushy stunt virus (TBSV) isolates and the BS-3 strain of TBSV: b = strain BS-3, c = control preparation, p = pepper isolate, t = tomato isolate. **A)** central well: BS-3 antiserum; **B)** central well: BS-3 antiserum absorbed with the Moroccan TBSV pepper isolate; **C)** central well: pelargonium leaf curl virus (PLCV) antiserum absorbed with the Moroccan pepper TBSV isolate; **D)** PLCV antiserum absorbed with TBSV strain BS-3.

DISCUSSION

Previous reports on TBSV indicated that this virus is not of significant economic importance. In Britain, TBSV has been isolated only twice in 30 yr from naturally infected tomatoes, and carnation Italian ringspot virus (CIRV), a strain of TBSV, has been found in only two carnation samples among several thousands tested during 15 yr (8). Lovisolo et al. (13) observed a very low rate of spread in their tests with petunia asteroid mosaic virus (PAMV). Only pelargonium leaf curl virus (PLCV), another TBSV strain, seems to be widespread in cultivated geraniums in Britain (8), and Bercks (3) reported an apparently widely distributed TBSV isolate in grapevines in Germany. The virus has also been isolated from naturally infected sweet cherry (*Prunus avium* L.) in Canada (2) and Czechoslovakia (1). In general, however, the natural distribution of TBSV is considered to be limited and its economic importance as fairly low (9, 16). In contrast, the situation in Morocco

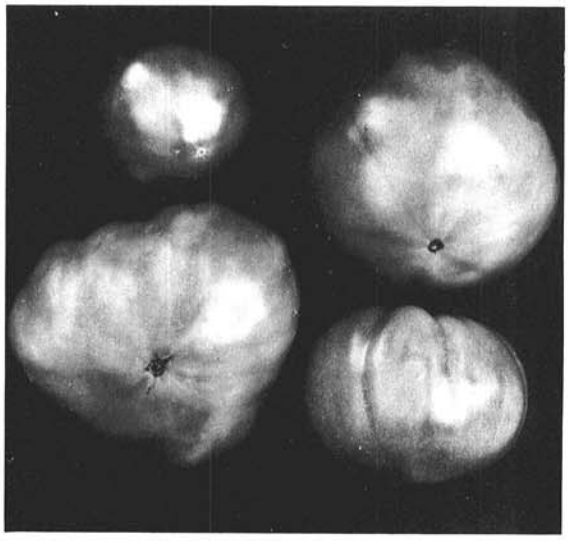

Fig. 3. Symptoms on mature tomato fruits naturally infected with the Moroccan tomato isolate of tomato bushy stunt virus (TBSV). Lighter, circular areas are yellow in color.

appears to be different: typical fruit symptoms of this disease were observed on large numbers of market tomatoes in April and May 1975 and again in 1976, and several tomato fields were found to be moderately infected. The only other instance of a significant field disease of tomatoes caused by TBSV was reported by Martinez et al. from Mexico (15).

In addition to the differential response produced on such test plants as *Chenopodium* spp., *Spinacia oleracea*, and *Datura metel*, the two Moroccan TBSV isolates were found to be two distinct serotypes. Previous reports have indicated that serological testing is more reliable than test plant reaction for differentiating between TBSV isolates or strains (4, 6, 18).

The precipitin lines of the tomato isolate always fused with those of the type strain (BS-3), and in reciprocal cross-absorption tests each virus eliminated all activity of the other. The Moroccan tomato TBSV isolate and the type strain must therefore be considered as closely related variants of TBSV, even though the Moroccan tomato TBSV isolate only occasionally produced systemic symptoms in tomato by mechanical inoculation and very rarely invaded *Datura stramonium* systemically.

On the basis of the immunodiffusion precipitin patterns obtained using unabsorbed and reciprocally cross-absorbed antisera, the pepper TBSV isolate was shown to be serologically distinct from the tomato isolate and type strain of TBSV. Based on the results of a cross-absorption test with PLCV antiserum it was concluded that the pepper isolate possessed more antigenic groups in common with this virus than with the tomato isolate and the type strain. Identity with PLCV, however, was excluded because of the inability of the pepper isolate to produce systemic symptoms in *Phaseolus vulgaris*, *Vigna unguiculata*, *Glycine max*, and *Datura stramonium* (6, 14). Pelargonium leaf curl virus in turn does not systemically infect tomato (4), and only causes symptomless local infection in *Spinacia oleracea* (6), a host which reacts both locally and systemically to the pepper virus. Serological tests were not sufficiently extensive to determine whether the Moroccan pepper TBSV isolate fell into any of the serological groupings of tombusviruses reported by Hollings and Stone (7).

Thornberry (17) does not list pepper among the plants infected by TBSV, and no subsequent mention of natural infection of this plant by TBSV was found in later literature. The properties of the Moroccan virus isolate from pepper do not appear to coincide with those of any previously reported strain of TBSV.

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