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Short communication. Effect of *Bemisia tabaci* biotype in the transmission of *Tomato Yellow Leaf Curl Sardinia Virus* (TYLCSV-ES) between tomato and common weeds

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Abstract

The common five weed species *Datura stramonium* L., *Solanum nigrum* L., *Brassica kaber* (DC), *Capsella bursa-pastoris* L. and *Malva parviflora* L., were tested for their susceptibility to an isolate of TYLCSV from Murcia, Spain (TYLCSV-ES), using the B-, Q- and S-biotypes of *Bemisia tabaci* (Gennadius). Both, B- and Q-biotypes were shown to transmit TYLCSV-ES from infected tomato to *S. nigrum* and *D. stramonium* and *vice versa*. Transmission efficiency from tomato to these weeds varied from 58.3% to 83.3%. Transmission efficiency from the infected weeds back to tomato varied from 66.7% to 100%. No significant difference between the B- and Q-biotypes was found in transmission efficiency from infected tomato to weed plants and from *D. stramonium* back to tomato. However, a significant difference in transmission efficiency from infected *S. nigrum* plants to tomato was detected between the B- and Q-biotypes. No other tested weed species were found to be infected by or host TYLCSV-ES. The S-biotype was unable to survive on tomato long enough to acquire or transmit TYLCSV-ES and could only transmit the virus from *S. nigrum* to *S. nigrum* at a very low efficiency. The implications of these results for the epidemiology of TYLCV´s in the field are discussed.

Key words: whiteflies, B biotype, Q biotype, S biotype, geminiviruses, TYLCV-Sar.

Resumen

Nota corta. Efecto del biotipo de *Bemisia tabaci* en la transmisión del virus del rizado amarillo del tomate (TYLCSV-ES) entre tomate y malas hierbas

Se analizó la susceptibilidad de las malas hierbas *Datura stramonium* L., *Solanum nigrum* L., *Brassica kaber* (DC), *Capsella bursa-pastoris* L. and *Malva parviflora* L. a un aislado del TYLCSV procedente de Murcia, España (TYLCSV-ES), utilizando como vectores los biotipos B, Q y S de *Bemisia tabaci* (Gennadius). Los biotipos B y Q transmitieron el TYLCSV-ES desde tomate infectado a *S. nigrum* y *D. stramonium* y viceversa, con una eficiencia de transmisión desde tomate a dichas malas hierbas entre el 58,3% y el 83,3%. La eficiencia de transmisión desde las malas hierbas al tomate varió desde el 66,7% al 100%. No se apreciaron diferencias significativas entre los biotipos B y Q respecto a la eficiencia de transmisión desde tomate a malas hierbas ni desde *D. stramonium* a tomate. No obstante, la eficiencia de transmisión desde plantas infectadas de *S. nigrum* a tomate fue significativamente diferente entre los biotipos B y Q. El aislado TYLCSV-ES no fue capaz de infectar a ninguna de las otras especies de malas hierbas utilizadas en este estudio. El biotipo S no pudo sobrevivir en tomate el tiempo suficiente para adquirir o transmitir el TYLCSV-ES, pudiendo solamente transmitir el virus desde *S. nigrum* a *S. nigrum* con una eficiencia muy limitada. Se discuten las implicaciones de estos resultados para la epidemiología en campo de los virus TYLCV.

Palabras clave: mosca blanca, biotipo B, biotipo Q, biotipo S, geminivirus, TYLCV-Sar.

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A TYLCD-causing virus was first reported in Spain in 1992 (Moriones *et al*., 1993), and this virus has since spread to all the main vegetable-producing regions of Southern and Eastern Spain. Isolates of the *Tomato Yellow Leaf Curl Sardinia Virus* (TYLCSV, previously known as TYLCV-Sar) and the Israeli strain of *Tomato Yellow Leaf Curl Virus* (TYLCV, previously known as TYLCV-Is), are now found to be coexisting within the Spanish tomato epidemics, and several isolates of both viruses from Murcia (actually TYLCSV-ES, following Fauquet *et al*., 2003) and Almería have now been sequenced (Moriones and Navas-Castillo, 2000).

Identifying weed plants that can serve as virus or vector reservoirs is of great importance within regions where tomato is grown seasonally. Some weed species have already been reported as reservoirs of whitefly-transmitted viruses (Bedford *et al*., 1998; Sánchez-Campos *et al*., 2000), particularly as a primary inoculum of TYLCV-like viruses (McGovern *et al*., 1994). Since some of common weeds are already known to be susceptible to some TYLCV's [*S. nigrum* to TYLCSV-AL (an isolate of TYLCSV) (Bedford *et al*., 1998) and *D. stramonium* to TYLCV (Cohen and Nitzany, 1966)], it is possible that they may also serve as reservoirs of TYLCSV-ES and therefore play a significant role in the epidemiology of this virus in the field.

Three biotypes of *Bemisia tabaci* (Gennadius) have so far been recorded within Spain, the B- and Q-biotypes (Guirao *et al*., 1997) and the S-biotype (Banks *et al*., 1999). The B- and the Q-biotypes are both responsible for transmitting and spreading viruses within Spanish agriculture, particularly in tomato crops. However, the S-biotype has only ever been recorded within a localised area near Malaga and only on *Ipomoea indica* (Banks *et al*., 1999). Muñiz (2000) and Nombela *et al.* (2001) have reported differences between the host responses to B- and Q-biotypes of *B. tabaci* in common weeds (*Datura stramonium* L., *Solanum nigrum*, L., *Brassica kaber* (DC), *Capsella bursa-pastoris* L. and *Malva parviflora* L.) and tomato plants. Moreover, some differences in transmission efficiencies of the *B. tabaci* biotypes when transmitting TYLCV/TYLCSV from tomato to tomato have been already reported (Sánchez-Campos *et al*., 1999), but few experimental studies had been undertaken to date on the transmission of TYLCSV-ES (Sánchez-Campos *et al*., 2000). Therefore, the aim of the present study was to compare the transmission efficiency of TYLCSV-ES by the Spanish B-, Q- and S-biotypes of *B. tabaci*, from different common weeds to tomato plants and *vice versa*.

The isolate TYLCSV-ES (Noris *et al*., 1994) was transmitted to tomato cv. Río Fuego by *B. tabaci*, and used as a virus source plant 30 days after inoculation. TYLCSV-ES infection was confirmed by symptoms and ELISA tests (see below). Colonies of two biotypes of *B. tabaci* identified by Guirao *et al*. (1997) as B and Q, were reared on virus-free tomato plants (cv. Río Fuego) within an insect-proof, virus-free chamber at 23:27°C (N:D) and 16:8 h (L:D) daylength. A colony of the S biotype was reared under similar conditions on healthy *I. indica* plants. Plants of *D. stramonium*, *S. nigrum*, *B. kaber*, *C. bursa-pastoris* and *M. parviflora* were grown in an insect-virus free chamber at 26 ± 1°C for 3-4 weeks and then tested as hosts of TYLCSV as described below. All weed plants were used at the «2 full leaf» stage as test plants.

Viruliferous whiteflies were obtained by caging ≈300 virus free *B. tabaci* adults on TYLCSV-infected tomato plants for a 72-h acquisition access period (AAP) (Mehta *et al*., 1994). Fifteen viruliferous whiteflies were then caged on each test plant for a 72-h inoculation access period (IAP) (Mehta *et al*., 1994). Whiteflies were then removed and the test plants sprayed with imidacloprid (Confidor-Bayer AG) at a concentration of 0.05%. Test plants were then placed in an insect-virus-free chamber for 3-4 weeks, and subsequently checked for TYLCSV infection by ELISA and PCR tests. In order to confirm whether virus from the infected weed plants could be transmitted back to healthy tomato plants, a back-assay transmission test was undertaken following the same procedure.

Ten replicates of the following groups of plants were used: 12 plants in the B-biotype virus transmission experiments from TYLCSV-infected weeds to tomato and *vice versa*, 12 plants in the experiments with the Q-biotype from infected *S. nigrum* weeds to tomato and 14 plants from infected *D. stramonium* weeds to tomato and *vice versa* in both cases.

TYLCSV was detected by ELISA and immunocapture-PCR, using the protocol described by Jiang *et al*. (2000).

Percentages (p) of transmission of TYLCSV were arcsin $p^{1/2}$ transformed and analysed with a one-way ANOVA and subsequently by the Tukey honest significant difference (HSD) test (StatSoft, 1994).

Fig.1 shows that both B and Q-biotypes of *B. tabaci* were able to transmit TYLCSV-ES from infected tomato plants to *S. nigrum* and *D. stramonium* and *vice ver-*

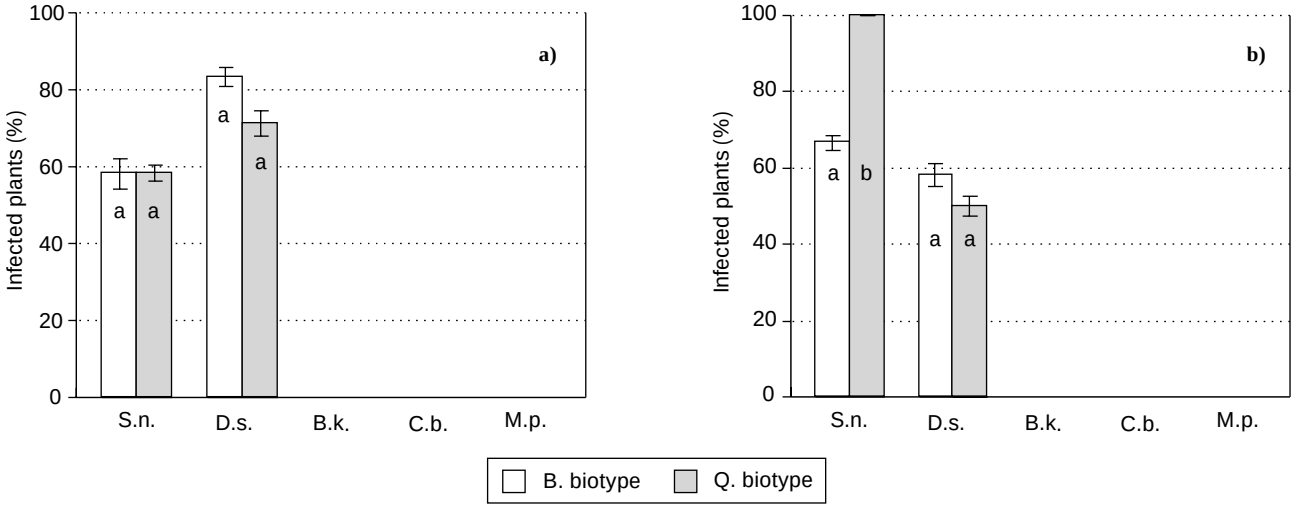

Figure 1. Averaged percentages of TYLCSV-ES transmission by B and Q biotypes of *Bemisia tabaci* from infected tomato to weed plants (a) and *vice-versa* (b). S.n.: *Solanum nigrum.* D.s.: *Datura stramonium.* B.k.: *Brassica kaber.* C.b.: *Capsella bursapastoris.* M.p.: *Malva parviflora.* Different letters within bars for the same weed species represent significant differences ($P < 0.05$) between biotypes. Error bars indicate the SEM.

sa. However, none of the other 3 weed species (*B. kaber*, *C. bursa-pastoris* and *M. parviflora*) were able to be infected by TYLCSV-ES. Moreover, no significant difference was found in transmission efficiency from infected tomato plants to weed plants between the B- and Q-biotypes. The S-biotype could not survive on tomato long enough to acquire or transmit TYLCSV-ES. However, this biotype could occasionally transmit the virus from *S. nigrum* to *S. nigrum* at very low efficiency (data not shown). The Q-biotype was subsequently shown to transmit TYLCSV-ES from tomato to *D. stramonium* with slightly less efficiency than the B-biotype (71.4% *vs.* 83.3%) (Fig 1). This was also the case for *D. stramonium* back to tomato plants (50.0% *vs.* 58.3%). Both biotypes were found to have the same transmission efficiency (58.3%) from tomato to *S. nigrum*. However, the transmission efficiency of the Q-biotype from *S. nigrum* back to tomato was significantly higher than the B-biotype (100% *vs.* 66.7%).

Our results strongly indicate that *B. kaber*, *M. parviflora* and *C. bursa-pastoris* are not hosts of TYLCSV-ES. Moreover, our transmission tests showed that *M. parviflora* could not be infected with TYLCSV-ES, regardless of whether B- or Q-biotypes were used. These results confirm that TYLCSV-ES is a different virus to TYLCV, having a different alternative host range and sequence data. However, there is a possibility that transmission efficiencies could be linked to a specific indigenous whitefly population that was not tested in this study. McGrath and Harrison (1995), for example, compared transmission of three different isolates of TYLCV using different *B. tabaci* cultures. The isolate of the TYLCD-associated virus from Nigeria could not be transmitted from tomato to tomato by whiteflies from the Ivory Coast but were transmitted by a population from Pakistan. They also showed that B-biotypes from the USA and Ivory Coast whiteflies transmitted the isolate of the TYLCD-associated virus from Senegal more efficiently than the isolate from India whereas Pakistan whiteflies transmitted the isolate from India more efficiently than the isolate from Senegal. These different transmission efficiencies by different *B. tabaci* populations appear to be associated with large differences in the coat protein epitope profiles of the virus isolates (McGrath and Harrison, 1995). Since we used a different TYLCD-associated virus and a different whitefly population in our study to those used by Cohen *et al*. (1988), it is not surprising that *M. parviflora* was not found to be infected. Additionally, Mansour and Al-Musa (1992) also excluded *M. parviflora* from the host list of TYLCV (isolate from Jordan Valley) in their host-range studies.

Although the S biotype is an almost monophagous biotype that was not able to survive on tomato, it was shown to be a vector of TYLCSV since the S biotype could occasionally transmit the virus from *S. nigrum* to *S. nigrum*. This was a reflection of its ability to survive longer on *S. nigrum* than on tomato as had previously been reported with an African *B. tabaci* population (Bedford *et al*., 1994). The reasons for the

different transmission efficiency of the Q-biotype from *S. nigrum* back to tomato are unclear, although it could be linked to the feeding behaviour of the different biotypes on different host plants. Whether this is because the different biotypes feed more voraciously on one plant than on another or whether they feed in different locations on or within the leaf (affecting virion acquisition), will require a further investigation. A difference in the transmission efficiency of TYLCV by *B. tabaci* biotypes has also been reported by Caciagli *et al*. (1995) and McGrath and Harrison (1995). The reproductive activity of B and Q-biotypes of *B. tabaci* was shown to differ (Nombela *et al*., 2001), and studies using the electrical penetration graph technique (EPG) on stylet penetration in relation to probing and feeding behaviour (Jiang *et al*., 2001) of these two *B. tabaci* biotypes also showed differences.

Since TYLCD-associated viruses cannot be controlled directly by the use of chemicals, control strategies have mainly focused on methods that prevent the occurrence of the disease ingressing a crop. Fully understanding the epidemiology of TYLCD is therefore crucial if effective and sustainable disease control programs are to be designed in the future. By eliminating infected weed plants that maintain a continued reservoir of TYLCD outside of a crop, the risk of that crop becoming infected will be reduced. This is especially so in the regions where tomato is grown seasonally. More extensive epidemiological studies combined with TYLCD-resistance breeding are therefore important components of future studies for developing these disease control strategies.

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