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TRANSMISSION PROPERTIES OF *TOMATO YELLOW LEAF CURL VIRUS* FROM TANZANIA

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Abstract: The tomato yellow leaf curl virus infecting tomato plants in Tanzania is reported to be different from the Old World geminiviruses. A study was initiated to investigate the transmission properties of the virus such as, acquisition feeding time, inoculation feeding time, persistence of virus in the vector, mechanical inoculation, seed and graft transmission. Results obtained indicate that the virus is transmitted persistently by *Bemisia tabaci* Genn., but neither mechanically – nor seed-transmissible. Minimum acquisition and inoculation time was 30 minutes. It is concluded that the properties of the agent causing the yellow leaf curl symptoms in tomato plants from different regions of Tanzania are similar and mimic those of tomato yellow leaf curl *Begomovirus* species studied elsewhere.

Key words: TYLCV, vector-host relationships, *Bemisia tabaci,* Tanzania

INTRODUCTION

Geminiviruses constitute a major threat to the production of tomatoes worldwide, especially, in the tropics and sub-tropics. *Tomato yellow leaf curl virus* (TYLCV) is a major threat to the tomato crop in many tropical and sub-tropical regions (Makkouk and Laterrot 1983). The virus accounts for huge losses in quantity and quality of tomatoes if unchecked. Incidences as high as 100% with undesirable consequences of crop failure have been recorded (Ioannou 1985; Pico et al. 1996).

The disease is one of the major virus diseases causing low yields of tomatoes in Tanzania, especially, in farmers´fields. Disease incidence of 100% had been reported in some regions in Tanzania mainland (Nono-Womdim et al. 1996; Kashina et al. 2003). The virus causing the yellow leaf curl symptoms on tomatoes in Tanzania is reported to be different from the Old World geminiviruses (Chiang et al. 1997). Detailed information on the transmission of the virus in Tanzania is lacking. Hence, a study was initiated to collect information on the transmission properties of the virus for effective

management of both vectors and the disease. The results are reported and discussed in this paper vis-à-vis reports on tomato yellow leaf curl virus strains reported elsewhere.

MATERIALS AND METHODS

Establishment and maintenance of virus culture

The virus cultures were established and maintained in the screenhouse at Sokoine University of Agriculture (SUA), Tanzania. Young tomato shoots with typical yellow leaf curl symptoms suggestive of TYLCV infection, were collected from tomato farms in six regions of Tanzania (Dodoma, Arusha, Kilimanjaro, Iringa, Coast and Morogoro), and grafted onto tomato seedlings, cv. Moneymaker. Subsequently, the virus cultures were maintained through whitefly-mediated inoculations. The regions were selected based on differences in TYLCV incidence reported by Nono-Womdim et al. (1996).

Establishment and maintenance of Bemisia tabaci colony

To establish the *B. tabaci* colony, the insects were reared in insect-proof cages following procedures described by the International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, modified from Rom et al. (1993). About 50 adult whiteflies were collected from field-grown tomato plants in Morogoro using a battery-operated aspirator. These were transferred into vials and chilled in the refrigerator (4°C) for 5–10 min. The chilled whiteflies were transferred to the leaves of young tomato seedlings maintained in insect-proof cages in the screenhouse, and confined in 5 cm-diameter clip cages on the underside of the upper leaves. After 2–3 h of confinement to allow for oviposition, the clip cages were removed and the eggs were allowed to hatch and develop on the plants.

Graft transmission

Each of the six viral isolates was wedge-grafted onto four-week old tomato seedlings cv. Moneymaker (stock). Five seedlings were grafted per viral isolate, and repeated twice. A longitudinal cut was made way down the stock using a sterile razor blade. The stem portion of the scion infected with the isolate was shaped into a wedge and inserted into the position of the cut made in the stock in such a way that there was close contact between the exposed tissues of both the scion and stock. Using a budding tape, the union was held in place making sure that no spaces existed between the two. The grafted seedlings were maintained in the screenhouse (25 ±5°C, 72.4% relative humidity) for 2 months to assess graft success, viral incidence and symptom severity for each isolate.

Vector transmission

Adult *B. tabaci* from the established non-viruliferous colony were allowed a 48-h access feeding period on the virus-infected tomato plants before being transferred in groups of 15 using an electronic aspirator to healthy seedlings of tomato cv. Moneymaker at the two-leaf growth stage for inoculation access feeding period of 24 h. The plants were monitored under screenhouse conditions (25 ±5°C, 72% relative humidity) after killing the whiteflies with a spray of Karate EC (a.i. λ-cyhalothrin). Symptoms of virus infection were recorded at weekly intervals for 4 weeks. The data on

virus transmission were used to estimate the percentage of whiteflies that transmitted (100 P) the virus from the formula of Gibbs and Gower (1960):

$$P = 1 - (1 - R/N)^{1/i}$$

where, P = the probability of a single whitefly transmitting the virus
R = number of infected plants
N = number of plants exposed to infection
i = number of whiteflies per test plant

Mechanical transmission

Mechanical transmission tests were conducted as described by Ioannou (1985). Infected leaf tissues collected from the uppermost leaves were homogenized in 0.01M phosphate buffer, pH 7.4 (1:10 w/v), 1.0% polyvinyl pyrrolidone (PVP-40). The sap was expressed from the homogenate and used to rub-inoculate leaves of test plants, which were pre-dusted with carborundum powder (500 mesh, 50 μm). Five plants each of tomato cv. Moneymaker, *Datura stramonium* L. and *Nicotiana tabacum* L. were tested three times. Non-inoculated plants were kept as controls. Both the inoculated and non-inoculated plants were maintained in the screenhouse for observation and assessment of disease incidence and symptom severity.

Seed transmission

Seeds were extracted from tomato fruits obtained from field- and graft-infected tomatoes by blending the fruits in distilled water and separating them from the debris through several steps of washing with distilled water. The seeds were sown in 5 plastic pots of 30 cm in diameter (10 seeds per pot), and maintained in the screenhouse.

Acquisition access period (AAP)

Adult non-viruliferous *B. tabaci* were allowed acquisition access feeding periods of 10, 30 min, or 1, 2, 4, 8, 16, 24 or 48 h and thereafter, transferred in groups of 15 to indicator plants for a 48-h IAP. The whiteflies were killed with Karate 5EC (a.i. 50 g/l λ-cyhalothrin) after inoculation feeding. Fifteen plants were used per test.

Inoculation access period (IAP)

Adult whiteflies were given AAP feeding of 24 h on tomato plants infected with each TYLCV isolate, and transferred to indicator plants for an IAP of 10, 30 min, or 1, 2, 4, 8, 16, 24 or 48 h. The same number of plants and whiteflies per plant was used as in AAP tests. The whiteflies were killed with Karate 5EC (a.i 50 g/l λ-cyhalothrin) at the expiration of each IAP.

Persistence of TYLCV in the vector

Three groups of 25 adult non-viruliferous *B. tabaci* were caged for 48 h on tomato plants infected with the TYLCV isolates. Thereafter, the viruliferous whiteflies were moved in daily serial transfers to healthy tomato plants. The indicator plants were sprayed with Karate 5EC (a.i. 50 g/l λ-cyhalothrin) after each transfer and monitored in the screenhouse for disease symptom development.

RESULTS

Graft transmission

The TYLCV was readily graft-transmitted to the test cultivar, Moneymaker. Initial symptoms developed within 2–4 weeks and the full syndrome within 1–2 months. Graft success of 80% was achieved with the Dodoma, Coast and Iringa isolates compared with Kilimanjaro (73.33%), Arusha (66.67%), and Morogoro (66.67%). However, all the test plants successfully grafted with the Dodoma and Morogoro isolates were infected (100%) with TYLCV 8 weeks after grafting compared with the Coast (91.67%), Arusha (90%), Kilimanjaro (81.81%) and the Iringa (75%) isolates. Similarly, TYLCV symptoms were most severe on plants graft-infected with the Dodoma (3.54) and Morogoro (3.12) isolates and least severe (1.68) on plants graft-inoculated with the Iringa isolate (Table 1).

Table 1. Comparative transmission efficiency of *Tomato yellow leaf curl virus* isolates by grafting

Virus isolate	Transmission			
	Rate[a]	Graft success [%][b]	Incidence [%][c]	Severity[d]
Arusha	9/10	66.67^{c}	90.0^{b}	2.56^{c}
Kilimanjaro	9/11	73.33^{b}	81.81^{c}	1.89^{d}
Dodoma	12/12	80.0^{a}	100.0^{a}	3.54^{a}
Morogoro	10/10	66.67^{c}	100.0^{a}	3.12^{ab}
Iringa	9/12	80.0^{a}	75.0^{d}	1.68^{d}
Coast	11/12	80.0^{a}	91.67^{b}	2.98^{bc}
CV (%)		2.02	2.24	4.16
SE		1.21	2.01	0.07

Means followed by the same letter within a column are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Tests

[a] number of plants infected /number of plants successfully grafted
[b] (number of plants successfully grafted/total number of plants tested x 100%
[c] (number of plants infected /number of plants successfully grafted) x 100%
[d] severity assessed based on scale of 0 (no symptoms) – 4 (very severe symptoms) (Friedmann et al. 1998)

Vector transmission

Transmission of the disease agent to healthy tomato plants was achieved using each of the test isolates. The Dodoma isolate was most efficiently transmitted followed by the Morogoro isolate. The two isolates were also the most severe, recording severity scores of 3.62 and 3.21, respectively. The Arusha and Iringa isolates recorded the least TYLCV incidence. However, the Iringa isolate was the least severe of all the test isolates (Table 2). All the insects transmitted the Dodoma isolate of the virus, while only 6.3% of the insects transmitted the Kilimanjaro isolate. The Morogoro isolate was transmitted by 12.45% of the whiteflies, while 9.70% and 7.73% of the insects transmitted the Coast, Arusha and Iringa isolates, respectively (Table 2).

Table 2. Comparative transmission efficiency of viral isolates by *Bemisia tabaci*

Virus isolate	Transmission		
	Rate[a]	Incidence[b] [%]	Severity[c]
Arusha	12/15	80.0 (7.73)	2.71
Kilimanjaro	11/15	73.33 (6.34)	2.0
Dodoma	15/15	100.0 (100)	3.62
Morogoro	14/15	93.33 (12.45)	3.21
Iringa	12/15	80.0 (7.73)	1.92
Coast	13/15	86.67 (9.70)	3.12

[a] number of infected plants/number of plants tested
[b] (number of infected plants/number of plants tested) x 100%, numbers in parenthesis refer to the percentage of insects that transmitted the virus
[c] severity asssed on a scale of 0–4 (Friedmann et al. 1998)

Mechanical transmission

Characteristic TYLCV symptoms did not develop on all the inoculated test plants. The addition of PVP-40 in the inoculation buffer to improve transmission did not result in mechanical transmission of TYLCV. To confirm these results, the test plants were back-indexed using *B.tabaci*, yet no TYLCV symptoms developed.

Seed transmission

Typical TYLCV symptoms were not observed on any of the tomato seedlings grown from seeds of infected plants, and no symptoms developed on indicator plants following back-indexing.

Virus-vector relationships

The minimum AAP for transmission of the virus isolates was 30 min except, for the Kilimanjaro and Iringa isolates, which were transmitted after acquiring the virus for a longer time of 1 h (Table 3). The vectors were able to transmit the six isolates of the virus to all the test plants after a 48-h access feeding on source plants. Similarly, the virus isolates were transmitted after 30 min following a 24-h AAP (Table 4). Thereafter, transmission increased with AAP and IAP. Transmission of the Dodoma and Morogoro isolates to all the test plants was achieved after an AAP and IAP of 24 and 16 h, respectively. The other virus isolates were transmitted to all the test plants when the insects were allowed an IAP of 24 h (Table 4).

After a 24-h AAP, the whiteflies retained the ability to transmit the virus for up to 11 days for the Dodoma and Morogoro isolates. However, there was a gradual decline in the number of infected plants after the fourth day, except for the Morogoro and Dodoma isolates, which started declining after the fifth day (Table 5).

Table 3. Effect of acquisition access time on the transmission of *Tomato yellow leaf curl virus* isolates

Virus isolate	Rate of transmission								
	Acquisition Access Time (AAP)								
	10 min	30 min	1 h	2 h	4 h	8 h	16 h	24 h	48 h
Arusha	0/15	1/15	3/15	6/15	6/15	9/15	12/15	12/15	15/15
Kilimanjaro	0/15	0/15	2/15	6/15	6/15	11/15	12/15	12/15	15/15
Dodoma	0/15	2/15	4/15	6/15	7/15	12/15	13/15	15/15	15/15
Morogoro	0/15	2/15	3/15	4/15	7/15	11/15	13/15	15/15	15/15
Iringa	0/15	0/15	2/15	5/15	6/15	8/15	11/15	11/15	15/15
Coast	0/15	1/15	3/15	5/15	6/15	9/15	12/15	12/15	15/15

Table 4. Effect of inoculation access period on the transmission of *Tomato yellow leaf curl virus* isolates

Virus isolate	Inoculation Access Time (IAP)								
	10 min	30 min	1 h	2 h	4 h	8 h	16 h	24 h	48 h
Arusha	0/15*	2/15	4/15	7/15	9/15	11/15	13/15	15/15	15/15
Kilimanjaro	0/15	2/15	3/15	5/15	8/15	9/15	12/15	15/15	15/15
Dodoma	0/15	2/15	5/15	8/15	10/15	13/15	15/15	15/15	15/15
Morogoro	0/15	2/15	4/15	7/15	9/15	12/15	15/15	15/15	15/15
Iringa	0/15	1/15	3/15	6/15	8/15	9/15	11/15	15/15	15/15
Coast	0/15	1/15	2/15	7/15	9/15	9/15	13/15	15/15	15/15

* number of tomato plants infected/tested

Table 5. Persistence of *Tomato yellow leaf curl virus* isolates in *Bemisia tabaci*

Virus isolate	Number of test plants infected out of five													
	1*	2	3	4	5	6	7	8	9	10	11	12	13	14
Arusha	3	5	5	5	4	4	2	2	1	1	0	0	0	0
Kilimanjaro	4	5	5	5	3	3	2	1	1	0	0	0	0	0
Dodoma	5	5	5	5	5	4	3	2	1	1	1	0	0	0
Morogoro	5	5	5	5	5	4	3	2	1	1	1	0	0	0
Iringa	4	5	5	5	4	2	2	1	1	0	0	0	0	0
Coast	5	5	5	5	4	3	2	1	1	0	0	0	0	0

* indicates days

DISCUSSION

The results indicated that the virus was successfully transmitted to healthy tomato plants by grafting as well as by the vectors. None of the isolates had disease incidence lower than 66%. The incidences recorded through transmission by the use of vectors were consistently higher than by grafting. This indicated that vector transmission being the natural means of transmitting the virus, was a more efficient way of transmitting the virus. The time taken for the union of the scion and the root stock to get establish, probably played a crucial role in the systemic movement of the virus. Moreover, the scions and the root stock could have been derived from tomato varieties bred from different parents with differing genetic reaction levels against the virus, thereby creating differences in the gene micro-climate while attempting to form a union. Consequently, initial recognition and subsequent establishment of the virus could have been affected and delayed. Conversely, with vector transmission, the virus was inoculated into the test plants without necessarily causing changes in the morphology of the plant.

The high incidence levels of the Dodoma and Morogoro isolates corresponded with observations made during field visits for sample collection. Apart from the very severe infection and high vector population observed on tomato plants in Dodoma and Morogoro, it was known (by interviewing the farmers) that the main tomato varieties grown were Moneymaker and Roma VF. TYLCV incidences of 100% and 95% had been reported, respectively, on these varieties (AVRDC 1994). Moreover, the use of the variety Moneymaker as assay host was compatible with the scions taken from the same Moneymaker. At Kilimanjaro and Arusha regions, the tolerant variety Tengeru 97 was most commonly cultivated on the farms visited, while at Iringa and Coast, a combination of Roma VF and Cal-J was common.

Graft transmission of TYLCV have been reported (Ioannou 1985). The differences in disease incidence and symptom severity that were observed among the viral isolates could have also been due to differences in the concentration of the virus in the scions at the time of grafting, physiological conditions of both scion and stock, and initial recognition activities in the infection build-up process between scion and stock.

Attempts to transmit the virus mechanically and through seed, failed. Similar results have been reported by Cohen and Nitzany (1996), Ioannou (1985) and Brown and Nelson (1988). These results corroborate previous reports that the yellow leaf curling symptoms observed on the virus isolates were caused by a non-mechanically transmissible agent. However, under artificial conditions, mechanical transmission of TYLCV to tomato from plants of *D. stramonium*, *N. glutinosa* and *L. pennellii*, was achieved at low rate, and only in few cases from tomato to tomato (Makkouk et al., 1979; Abdel-Salam 1990). Cytopathological changes associated with TYLCV infections have been observed in the nuclei of phloem parenchyma, companion cells and differentiating sieve tubes (Cherif and Russo 1983; D'Hondt and Russo 1985). This phloem localization was thought to be responsible for the difficulty in mechanical transmission, which distinguishes TYLCV from other bipartite geminiviruses capable of invading the mesophyll tissues (Wege et al. 1994). The TYLCV studied exhibited this difficulty.

The minimum AAP was 30 min for each of the TYLCV isolates used, except for the Kilimanjaro and Iringa isolates, which had a minimum AAP of 1 h. However,

all the TYLCV isolates tested had a minimum IAP of 30 min. A minimum AAP of 1 h was reported for *Tomato leaf crumple virus* (ToLCrV), a whitefly-transmitted virus (Brown and Nelson 1988). Mansour and Al-Musa (1992), reported a minimum AAP of 1 h for TYLCV. The increase in the number of infected plants with increasing AAP, suggests that a possible dose response relationship existed between the vectors and TYLCV isolates. It could also mean more females were involved in transmitting the virus than males.

The ability of the whiteflies to retain the virus for 10–11 days after acquisition, was an indication of a persistent type of relationship between the virus and the host. Similar observations have been reported elsewhere (Cohen and Nitzany 1966; Rubinstein and Czosnek 1997). *Tomato yellow leaf curl virus* is transmitted in a persistent and circulative manner by the whitefly vectors. Antignus et al. (1994) reported that the coat protein (CP) was only retained in the vector for 10 days after an AAP of 48 h. This means that the CP influences vector infectivity since viruliferous vectors have been reported to remain infective for 10–12 days (Ioannou 1985; Mansour and Al-Musa 1992).

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POLISH SUMMARY

WŁAŚCIWOŚCI TRANSMISYJNE WIRUSA ŻÓŁTEJ KĘDZIERZAWOŚCI POMIDORA Z TANZANII

Stwierdzono, że wirus żółtej kędzierzawości pomidora infekujący rośliny pomidora w Tanzanii różni się od geminiwirusów Starego Świata. Badania podjęto w celu określenia właściwości transmisyjnych tego wirusa, takich jak: czas żerowania wektora na roślinie porażonej potrzebny do nabycia przez niego wirusa, czas żerowania wektora na roślinie infekowanej potrzebny do jej inokulacji, czas życia wirusa w wektorze, inokulacja mechaniczna oraz przenoszenie przez nasiona lub przeszczepianie. Otrzymane wyniki wykazały, że wirus jest przenoszony przez *Bemisia tabaci* Genn., nie jest natomiast przenoszony mechanicznie ani przez nasiona. Minimalne czasy nabycia wirusa przez wektora i inokulacji rośliny wynosiły 30 minut. Zatem właściwości czynnika powodującego objawy żółtej kędzierzawości na pomidorach z różnych regionów Tanzanii są podobne i nie odbiegają od właściwości garunków *Begomowirus* badanych gdzie indziej.