

SHORT COMMUNICATION

Pyramiding TYLCV and TSWV resistance genes in tomato genotypes

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ABSTRACT: The Cuban tomato line LD3, in which the *Ty-1* gene from *Solanum chilense* LA 1969 was detected, was crossed with an F₁ hybrid carrying the genes *Ty-3* from *S. chilense* LA 2779 and *Sw-5* from *S. peruvianum*. A hundred F₂ plants were transplanted to a ferralitic soil in the field and the F₂ plants individually evaluated in the field with high natural TYLCV-IL (Cu) incidence by using a scale from 0 (no symptom) to 4 (severe stunting). The total DNA extracted from the F₂ plants were analyzed per individual plant by using PCR markers linked to the genes *Ty-3* (resistance to TYLCV) and *Sw-5* (resistance to TSWV) so that both genes could be detected in a same genotype. Eighty two F₂ plants showed no symptoms under natural TYLCV incidence (0 symptoms) with a productivity ranging from 1.6 to 9.7 kilograms per plant. The Principal Component Analysis (PCA) permitted grouping the different genotypes. Six F₂ plants showed the *Ty-3* gene in homozygosis and 12 in heterozygosis; 33 F₂ plants showed the *Sw-5* gene according to their DNA analysis. Three F₂ plants (number 65, 68 and 78) showed the *Ty-3* and *Sw-5* genes in homozygosis besides the *Ty-1* gene (resistance to TYLCV) from their female parent. Their productivity ranged from 6.0 to 6.8 kilograms per plant. These are the first Cuban results in which resistance genes for two important diseases in the tomato crop are obtained in a same genotype by the early and simultaneous molecular screening for resistance and the productivity evaluation under field conditions. Both tools, when simultaneously used, are efficient in reducing selection time and space.

Key words: TYLCV, TSWV, pyrimiding, resistance genes, tomato genotypes.

Pirimidación de genes de resistencia a TYLCV y TSWV en genotipos de tomate

RESUMEN: En este estudio, los cruzamientos se realizaron entre la línea cubana LD3, con gen de resistencia *Ty-1*, procedente de *Solanum chilense* LA 1969, y el híbrido F₁ portador de los genes *Ty-3* de *S. chilense* LA 2779 y *Sw-5* de *S. peruvianum*. Se obtuvieron 100 plantas F₂ que fueron trasplantadas a campo, en suelo ferralítico rojo. Las plantas F₂ fueron evaluadas en condiciones naturales de campo en áreas con alta presión de TYLCV-IL (Cu). Las plantas individuales fueron evaluadas con escala de síntomas desde 0 (sin síntomas) a 4 (enanismo severo). Los ADN totales extraídos fueron analizados en plantas individuales de la población F₂ en estudio, utilizando marcadores PCR ligados a los genes *Ty-3* (resistencia a TYLCV) y *Sw-5* (resistencia a TSWV) con el objetivo de detectar estos genes en un mismo genotipo. Un total de 82 plantas F₂ no mostraron síntomas bajo condiciones naturales de incidencia de TYLCV-IL (Cu) (grado 0) con un rango de productividad de 1.6 a 9.7 kilogramos por plantas. El Análisis de Componentes Principales permitió agrupar los genotipos. En seis plantas se detectó la presencia de gen *Ty-3* en homocigosis y en 12 plantas en heterocigosis; 33 plantas F₂ demostraron el gen *Sw-5* a partir de los análisis de ADN realizado. En tres plantas F₂ (números 65, 68 y 78) se encontraron en homocigosis los genes *Ty-3* y *Sw-5* en adición del gen *Ty-1* (resistencia a TYLCV) procedente del parental femenino. El rango de productividad obtenido fue de 6.0 a 6.8 kilogramos por plantas. Estos son los primeros resultados en Cuba donde se obtienen genes de resistencia en un mismo genotipo para dos importantes enfermedades del cultivo del tomate con una evaluación simultánea y temprana de la resistencia y la productividad en condiciones de campo, mediante el uso temprano de marcadores moleculares en la reducción del tiempo y el espacio en el proceso de selección de genotipos promisorios.

Palabras clave: TYLCV, TSWV, pirimidación, genes de resistencia, genotipos, tomate.

Begomoviruses, mainly the *Tomato yellow leaf curl virus* (TYLCV), and tospoviruses, such as *Tomato spotted wilt virus* (TSWV), *Tomato Chlorotic spot virus* (TCSV) and *Groundnut ring spot virus* (GRSV), are major diseases in the tomato (*Solanum lycopersicum* L.) crop. In Cuba, TYLCV became the most important tomato disease since the last eighties when it appeared in the country (1). On the contrary, tospovirus species has not been reported in the Island, but it is a major constraint in different neighboring countries.

Different TYLCV resistance genes have been reported to provide a broad spectrum of protection to many begomovirus species that are wide spread in the world (2). Zamir *et al.* (3) were the first to report a major incompletely dominant gene (*Ty-1*) on chromosome 6 derived from *Solanum chilense* (Dunal) Reiche LA 1969. Plants homozygous for *S. chilense* alleles at TG297 and TG97 markers on chromosome 6 were symptomless, while susceptible control had severe symptoms. The introgression of this gene provides high resistance to a broader array of begomoviruses to some Cuban parent lines (4). Ji and Scott (5) reported a partially dominant gene also on the long arm of chromosome 6, further designated *Ty-3* gene (6, 7) in lines derived from *S. chilense* LA2779. The gene *Sw-5* confers resistance to TSWV in a dominant way (8)

A breeding program was needed at the Horticultural Research Institute «Liliana Dimitrova» (IIHLD) supported by the National Center for Animal and Plant Health (CENSA), to improve resistance level by pyramiding genes for TYLCV and TSWV resistance in high yielding genotypes to enhance a sustainable production. The aim of the present work was to incorporate different TYLCV resistance genes (*Ty-1* and *Ty-3*) and the *Sw-5* gene (resistance to TSWV) into a single tomato genotype to be screened by molecular markers in early generations and to establish the relationships between TYLCV resistance and productivity traits.

The cross of the genetic material was made between LD3, a Cuban tomato line in which the *Ty-1* gene from *S. chilense* LA 1969 was detected by Piñón *et al.* (9), and an F_1 hybrid carrying the *Ty-3* gene from *S. chilense* LA 2779 and the *Sw-5* gene from *S. peruvianum*. In a field trial, 100 F_2 plants were transplanted to ferralitic soil at the IIHLD. The distance between the plants was 0.25 m with an interrow space of 1.40 m, respectively. No chemical treatment was applied to control *Bemisia tabaci* Genn., the TYLCV-vector.

After transplanted, the F_2 plants were evaluated under high natural TYLCV incidence in open field conditions with no escape to the susceptible control

cv. 'Campbell 28'. The plants were individually evaluated weekly until 56 days after inoculation by using a scale from 0 (no symptom) to 4 (stunting) (10).

The plant DNA was extracted using a short nucleic acid extraction technique (11). Leaf tissue (0.15 g) was macerated in liquid nitrogen followed by the addition of the extraction buffer (100 mM Tris-HCL pH8, 50 ml NaCl, 1% SDS, 10 ml β -mercaptoethanol). The final product was suspended in 300 μ l of TE buffer (10 mM Tris-HCL, pH8 1 ml EDTA). The total DNA extracted from the F_2 plants were analyzed per individual plant by using PCR markers linked to the genes *Ty-3* (7) and *Sw-5* (8) so that both genes could be detected in a same genotype. The individual plants carrying these genes were quantified. The PCR-fragments were visualized with UV light and purified from PCR reaction according to the standard protocol (Wizard Plus SV miniprep DNA purification System). The sequencing was conducted under BigDye™ terminator cycling conditions (Macrogen Service). The Correlation and Principal Component analyses were carried out using SAS 8.1 software (SAS Institute, Cary, N. C. USA), in which variables such as TYLCV severity, number of flowers, fruits and fruit set at the first four bunches, number of bunches and number of total fruits per plant; mean fruit weight and yield per plant were included.

Differences for TYLCV severity and for productivity characters were detected among genotypes. Eighty two F_2 plants showed no symptoms under natural TYLCV incidence (0 symptoms) with a productivity ranging from 1.6 to 9.7 kilograms per plant making possible to select for TYLCV resistance and high productivity. (Table 1)

TYLCV severity was negatively and significantly correlated with the flower number at the first four bunches in the plant ($r = -0.783$). It also decreased other productivity traits such as: fruit and bunch numbers per plant ($r = -0.886$ and -0.8334 , respectively), fruit set ($r = -0.93$) and yield per plant ($r = -0.893$), which explained the productivity decrease observed in this work when TYLCV severity increased (table 1). Gómez *et al.* (9) informed these effects in comparing resistant and susceptible tomato cultivars in tropical conditions. Mejias *et al.* (12) found that TYLCV infection led to yield loss mainly due to the reduction of the number of fruits per plant.

According to the Principal Component Analysis (PCA), where the first three components extracted 60% of the total variability, the genotypes could be grouped. The F_2 plants numbers 65, 68 and 78 were included into the 1st group highlighting for symptomless and high yield (6.0 to 6.8 kilogram per plant).

TABLE 1. Trait ranges of F₂ plants under natural TYLCV-IL (Cu) incidente./ *Rango de caracteres en plantas F₂, bajo la incidencia de infección natural de TYLCV-IL (Cu).*

TYLCV severity	No.of plants	Fruit set (%)	No. of bunches per plant	Fruit		
				No. per plant	Yield (Kg/ plant)	Weight (g)
0	82	60-100	8-21	10-35	1.6-9.7	128-375
1	15	54-100	6-15	8-29	1.3-5.3	124-325
2	1	54	5	6	0.7	120
3	2	53-80	5	6	0.70-0.72	110-120
4	0	-	-	-	-	-

Six F₂ plants showed the *Ty-3* gene in homozygosis with an amplified fragment of aprox. 640pb; twenty in heterozygosis and an amplified fragment of aprox. 500 and 640pb (Figure 1A), and 33 F₂ plants showed the *Sw-5* gene according to DNA analyses (Figure 1B) .

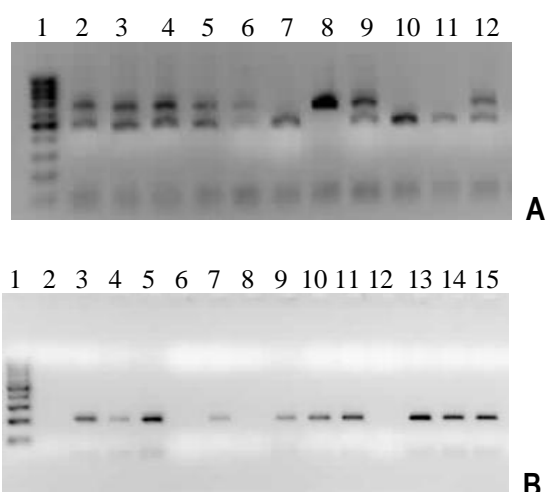


FIGURE 1 A y B. Genotypes analyzed using DNA markers A) Detection loci from *Ty-3* genes, line 8 in homozygous level, line 2-6, 9 in heterozygous level and line 12 positive control from parent. B) Detection loci from *Sw-5*, positive amplification in lines 3-5; 7, 9-11 and 13-14, line 15 positive control from parent./ *Genotipos analizados usando marcadores ADN. A) Detección del loci del gen Ty-3, línea 8 en homocigosis, líneas 2-6, 9 en heterocigosis, línea 12 control positivo de parentales. B) Detección del loci del gen Sw-5, amplificaciones positivas en líneas 3-5, 7, 9-11, 13 y 14, línea 15 control positivo del parental*

The sequence in forward and reverse direction of the 629 pb from the fragment showed 100% of identity with *Ty-3* gene from Gc43 Genotype resistant to begomoviruses in Guatemala (12).

The transfer of the gene *Sw-5* was observed for the amplification of PCR-fragments about 200pb with 100% of identity to *Sw-5a* gene from Genebank (AY007366.1).

Three F₂ plants (number 65, 68 and 78), symptomless and with high productivity, showed the *Ty-3* and *Sw-5* genes in homozygosis besides the *Ty-1* gene (resistance to TYLCV) previously reported by Piñón *et al.* (2) in their female parent.

DNA marker technology has been used in commercial plant breeding programs since the early 1990's and has proved helpful for rapid and efficient transfer of useful traits into agronomically desirable varieties and hybrids. In this work the main goal was achieved through the early molecular screening for resistance and the productivity evaluation under field conditions; both tools, when simultaneously used, showed to be efficient in reducing selection time and space.

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