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Original Article

Resistance to *tomato yellow leaf curl Thailand virus*, TYLCTHV-[2] from *Solanum habrochaites* accession 'L06112' in F_1 and BC_1F_1 generations

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Abstract

Resistance to tomato yellow leaf curl disease caused by *Tomato yellow leaf curl Thailand virus* (TYLCTHV-[2]) in wild tomato, *Solanum habrochaites* 'L06112' was investigated. The 'L06112' accession expressing the resistant phenotype was crossed to the TYLCV-susceptible female parent, Seedathip3, to produce F_1 hybrids. Parental polymorphism and hybrid identity were tested using 12 pairs of microsatellite markers for each chromosome. All markers were polymorphic between the parents, but only markers SSR46, SSR115, SSR117 and SSR128 gave results suitable to assess hybrid relationships. Polymorphic bands were sharp, concise and distinguishable between hybrids and selfed plants. The stem cuttings of donor and recurrent parents, their F_1 and BC_1F_1 were inoculated with TYLCTHV-[2] using viruliferous whiteflies. Disease response of the plants was evaluated by Enzyme-Linked Immunosorbent Assay (ELISA) at 45 days post inoculation. The donor parental line showed complete resistance to TYLCTHV-[2] while the F_1 and BC_1F_1 expressed various ELISA readings for TYLCTHV-[2] concentration. BC_1F_1; 04T105-7, 04T105-1, 04T105-10, 04T109-4 and 04T104-1 developed from this study showed the high level of resistance to TYLCV, Thailand isolate.

Keywords: *Solanum habrochaites*, *S. lycopersicum*, Tomato yellow leaf curl disease, TYLCTHV-[2], Breeding for disease resistance

1. Introduction

Tomato, *Solanum lycopersicum* L. (formerly *Lycopersicon esculentum* Mill.) belongs to the Solanaceae family. One of the most significant problems in tomato production worldwide, especially in those areas where tomatoes are grown commercially, is tomato yellow leaf curl disease caused by *Tomato yellow leaf curl virus* (TYLCV). This disease was first observed and documented in the Middle East in 1960 (Cohen and Harpaz, 1964) and first observed in Thailand in 1974 (Sutabutra, 1989). Typical symptoms of this disease are yellowing and curling of the leaf margins with distinguishable venal chlorosis. This disease is considered systemic and will spread to the other tomato plants through whitefly (*Bemesia tabaci*) in a persistent manner.

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Virus transmission does not occur by eggs, plant seed or mechanical means. However, the virus remains viable in the whiteflies throughout their lifespan (Cohen and Nitzany, 1996; Rubinstein and Czosnek, 1997). Serious infections will result in stunting with small curled leaves, premature flower drop and embryo abortion resulting in 100% loss of fruit production (Lapidot *et al.*, 1997).

TYLCV is a circular single-stranded DNA plant virus of the genus *Begomovirus* in the *Geminiviridae* family with quasi-isometric particles. TYLCV has two types of genomes, a monopartite single molecule of DNA which dominates and a bipartite comprised of DNA-A and DNA-B. The bipartite genomes have been documented in India and Thailand (Czosnek and Laterrot, 1997).

The results from previous studies have shown several wild species of tomato with genetic resistance to TYLCV including *S. peruvianum, S. pimpinellifolium, S. cheesmaniae* and *S. habrochaites* (Scott *et al.*, 1995). It is believed that the genes responsible for resistance may be controlled by 1 to 5 genes that are either recessive or dominant depending upon the geographic origin of the particular species (Vidavsky and Czosnek, 1998). This study focused on genetic improvement of the Thai cultivar (Seedathip3) by means of introducing genes from a wild type tomato, *S. habrochaites* accession 'L06112' through conventional breeding. This breeding project was complemented by molecular marker technologies using DNA simple sequence repeat (SSR) to determine polymorphisms between both species and confirm their offspring identity.

2. Materials and Methods

2.1 TYLCTHV-[2] and whitefly cultures

TYLCTHV-[2] was maintained on 30-day old susceptible tomato cultivar-Seedathip3 and was propagated by grafting to the new susceptible seedlings regularly. Infected plants showed viral disease symptoms in new shoots 7 days after grafting and developed full disease symptoms in 14 days. Whiteflies were reared on eggplants in a separate greenhouse to avoid contamination.

2.2 Whitefly-mediated inoculation

Virus-infected tomato plants were placed with whiteflies in an insect-rearing greenhouse and whiteflies were allowed to feed for one week prior to each trial. Ten cuttings of each line were used as replications and placed in the greenhouse with viruliferous whiteflies and infected plants. Shoot samples were collected 45 days after the inoculation period and quantified for viral accumulation using the ELISA technique (Ganjadana *et al.*, 2002). The color reaction was measured as absorbance (optical density=OD) at 405 nm on an ELISA plate reader (Multiskan EX, Themo Labsystems OY, Finland). Data were analyzed using statistical analysis program and Duncan Multiple Range Test (DMRT) for mean separations at $p \leq 0.05$.

2.3 Breeding design

Solanum habrochaites accession no. L06112 from the Asian Vegetable Research and Development Center (AVRDC) and *S. lycopersicum* var. Seedathip3 were crossed to produce F_1 progenies. Twenty one F_1 hybrids were crossed back to a susceptible Seedathip3 to generate a BC_1F_1 generation because they failed to produce viable seed for an F_2 generation. Stem cuttings of *S. habrochaites,* Seedathip3, F_1 and BC_1F_1 generations were introduced to TYLCTHV-[2] by viruliferous whitefly inoculation as the flow chart described in Figure 1.

2.4 Parental polymorphisms and hybrid identification using microsatellite markers:

DNA from *S. habrochaites*, Seedathip3 and F_1 hybrids were extracted according to the methods described by Fulton *et al.* (1995); then, DNA samples were amplified using the Polymerase Chain Reaction (PCR) technique. PCR mixtures (20µl total volume) with forward and reverse primers; SSR 117, SSR66, SSR290, SSR94, SSR115, SSR128, SSR45, SSR15, SSR155, SSR248, SSR46 and SSR20 were used to probe each chromosome 1 to 12, respectively. Amplification conditions were 35 cycles of DNA melting, annealing and extension of 1min at 92°C, 1 min at their annealing temperature and 2 min at 72°C chronologically (source: http://www.sgn.cornell.edu/). Amplified DNA fragments were subjected to electrophoresis at 50°C, 80 watts for 90 min in 4.5% denaturing polyacrylamide gel (acrylamide:bis-acrylamide = 19:1, 7.5 M urea and 1XTBE buffer; 0.045M Tris-borate, 0.01M EDTA at pH8.0). Gels were fixed using 10% acetic acid for 20 min and rinsed 3 times, 10 min apart with distilled water, then stained in silver nitrate solution (0.1 % silver nitrate and 0.05% formaldehyde) for 30 min and quickly rinsed (0.5 min) with distilled water. Gels were developed by soaking

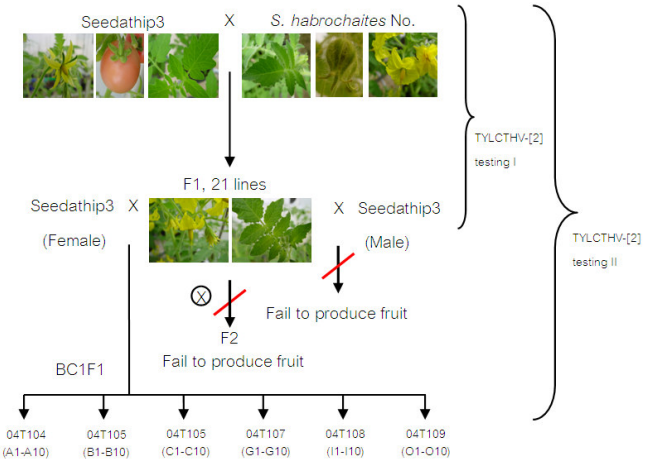

Figure 1. The crosses for tomato breeding lines between Seedathip3 and *S. habrochaites* accession no. L06112.

in chilled developer (3% sodium carbonate, 0.05% formaldehyde and 0.01% sodium thiosulfate) until the bands appeared with sufficient intensity. The reaction was stopped by adding 10% acetic acid and rinsed again in distilled water. Gels were dried at room temperature and DNA bands were viewed and scanned. The fixing, staining and developing processes were subjected to slow agitation on a shaker.

3. Results and Discussion

Improvement of tomatoes by exploiting traits from wild species is a slow process because of the complexity of the genes and linkage drag. However, there were several attempts reported on introgression of valuable traits from wild species, even though time requirements could be a discouraging factor for plant breeders. *Solanum habrochaites* is a wild tomato species that contains useful genetic resources for several diseases and insects (Rick and Chetelat, 1995). Accession no. L06112 from AVRDC was chosen for a breeding program for TYLCTHV-[2] resistance by crossing to a recurrent susceptible commercial tomato cultivar, Seedathip3 in Thailand. Only 53.33% of the F_1 hybrids between *S. habrochaites* 'L06112' and Seedathip3 set fruit (Whankaew *et al.*, 2005). Twenty one F_1 hybrids were planted to produce the F_2 generation but did not bear fruit. Therefore, the F_1 plants were reciprocally crossed back to their parents. Unfortunately, plants only set fruit when using Seedathip3 as the female parent (Figure 1). Similar results have been reported that interspecific hybridization using *S. habrochaites* as a male parent. *S. habrochaites* is a self-incompatible species in the *Eriopersicon* subspecies that can produce fruit only when crossed to self-compatible *Eulycopericon* subspecies (Allard, 1660; Kaloo, 1993; Shivanna, 2003 and Taylor, 1986).

As there are differences in morphology between these species, these differences were expressed in the progenies as well. DNA size polymorphisms from 12 mapped simple-sequence repeats (SSR) revealed the differences in the parents for each chromosome. All 12 pairs of markers were polymorphic between *S. lycopersicum* var. Seedathip3 and *S. habrochaites* accession no. L06112 (Figure 2). The differences in the patterns of DNA bands may be attributed to the number of SSR repeats between species or the specificity of the primers to either the male or female parent. This indicated that parental genomes of these two species of tomatoes are not closely related.

Selected primers in this study were successfully used to determine the differences between Seedathip3 and *S. habrochaites* 'L06112'; however, several primers were determined to be inadequate to assess hybrid identification. Marker SSR66 amplified only the bands of DNA from the female parent; therefore, this pair of primers cannot be used to distinguish the differences between a self and a cross using Seedathip3 as the female parent. Primers SSR15, SSR20, and SSR290 amplified DNA fragments of the same sizes but differed in the number of bands thus complicating hybrid

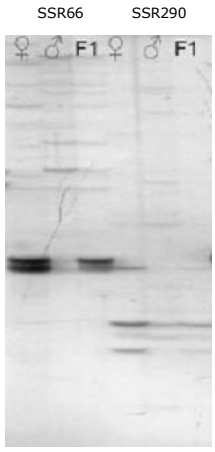

Figure 2. The polymorphic bands amplified by SSR primers revealing results of a cross between *Solanum lycopersicum* var. Seedathip3 ( ), *S. habrochaites* accession no. L06112 ( ) and their F_1 hybrids. SSR66 amplified only fragments from Seedathip3, while SSR290 amplified multiple bands at same sizes.

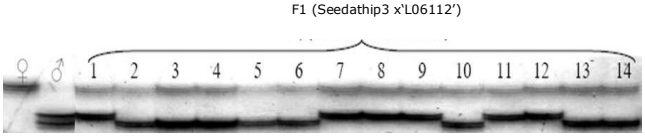

Figure 3. DNA bands of *Solanum lycopersicum* var. Seedathip3 ( ) and *S. habrochaites* accession no. L06112 ( ) and representative of F_1 generations using markers SSR115 in hybrid identification

identification. Hybrids receiving DNA from both female and male parents could possibly have the same DNA banding pattern as offspring derived from selfing. Although primers SSR94, SSR45 and SSR48 showed size polymorphisms in parents, they produced faint bands that were not rigorous enough to be definitive for line identity. The reliable primers that produced a sharp and concise banding for identification of crosses were SSR46, SSR115 (Figure 3), SSR117 and SSR128.

These polymorphic microsatellite loci are valuable for future use in tomato breeding programs. This information is also deemed important for the contribution to the expanding database used for chromosome mapping and marker assisted selection in tomato breeding. By knowing the reliable primers to use and thus obtaining definitive results, we can further test lines of tomatoes with confidence and improve the selection process.

The statistical analysis of the TYLCTHV-[2] screening test for *S. habrochaites*, Seedathip3 and their F_1 progenies showed a significant difference between the parents (table 1). Seedathip3 expressed the symptoms at the second week after inoculation and full, severe symptoms developed after 2 weeks. The ELISA reading (1.407) indicated a high level of virus present at 45 days post-inoculation. Conversely,

Table 1. Response of Seedathip3, *S. habrochaites* 'L06112 and their F1 generation for the presence of TYLCV 45 days after inoculation using ELISA technique. Means with different letters are significantly different at P≤0.05 according to the Duncan Multiple Range Test.

Phenotypes	ELISA reading with DMRT
SD3	1.407[a]
T	0.924[b]
W	0.855[bc]
P	0.853[bc]
J	0.843[b-d]
M	0.840[b-d]
U	0.710[b-d]
H	0.709[b-d]
L	0.673[b-e]
E	0.620[b-e]
V	0.579[b-e]
D	0.429[b-e]
S	0.400[b-e]
G	0.391[b-e]
K	0.389[b-e]
F	0.380[b-e]
N	0.329[c-e]
I	0.326[c-e]
C	0.268[de]
B	0.235[de]
A	0.125[e]
O	0.115[e]
L06112	0.014[e]

S. habrochaites did not show any symptoms through the period of inoculation and the ELISA reading was 0.014. Hybrids revealed intermediate symptoms and virus titer compared to the parents (Figure 4).

Because the F_1 did not set fruit, the most resistant five hybrids (A, B, C, I and O) and one hybrid (G) showing an intermediate response were chosen for further introgression. The resistant and tolerant BC_1F_1 plants were selfed. Only genotypes that set fruits and produced mature seeds were screened for TYLCV. The responses of each line including *S. habrochaites*, Seedathip3, F_1 and BC_1F_1 to TYLCTHV-[2] inoculation are shown in Table 2. These genotypes segregated for virus susceptibility, tolerance and resistance (Figure 5). *S. habrochaites* was symptomless and had the lowest ELISA reading at 0.069. The F_1 also had low readings varying from 0.072 to 0.188 for viral presence except for line G, which showed a higher reading from those in the previous test. Several individuals derived from B showed the best performance for virus tolerance among the comparative genotypes in the BC_1F_1 generation.

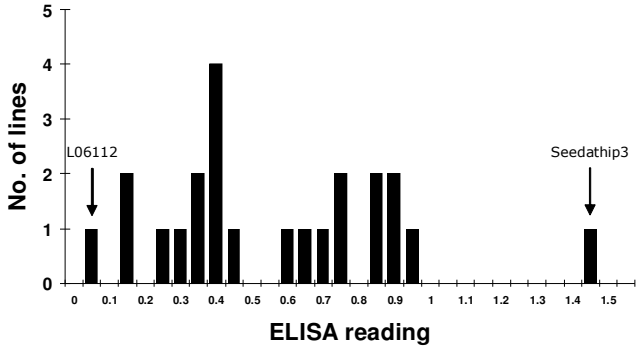

Figure 4. Distribution of tested tomato lines in Seedathip3, *S. habrochaites* 'L06112' and their F_1 using the ELISA detection of *Tomato Yellow Leaf Curl Virus*, Thailand isolate at 45 days post-inoculation.

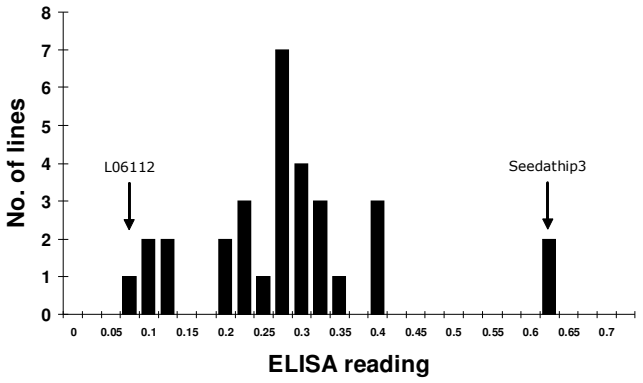

Figure 5. Distribution of tested tomato lines in Seedathip3, *S. habrochaites* 'L06112' and their BC_1F_1 using the ELISA detection of *Tomato Yellow Leaf Curl Virus*, Thailand isolate at 45 days post-inoculation.

Several accessions from *S. habrochaites* have been used in breeding programs for resistance to tomato yellow leaf curl disease. Research in Jordan using *S. habrochaites* 'LA386' identified that TYLCV resistance is controlled by more than a single dominant gene (Hassan *et al*. 1984). 'LA1777' was reported to be resistant to TYLCV-Cyprus isolate (Ioannou, 1985), but displayed tolerance to TYLCV from Sardinia and Senegal and ToLCV from India (Fargette *et al*., 1996). Vidavsky and Czosnek (1998) developed a tolerant TYLCV tomato line by crossing *S. habrochaites* 'LA386' and 'LA1777' and then introgressed this into a domesticated tomato *S. lycopersicum*. The BC_1F_4 generation showed tolerance to TYLCV-Israel. In 1990, Kalloo and Benerjee developed TYLCV resistant genotypes from *S. habrochaites* f. *glabrarum* accession 'B6013'. Resistance was expressed to TYLCV at AVRDC, Taiwan and to ToLCV in Bangalore, India. One of the lines, H24 was found to be controlled by a single gene, *Ty-2,* and mapped on chromosome 11 (Hanson *et al*., 2000). From this study, a donor parent line, *S. habrochaites* accession 'L06112' from the AVRDC showed complete resistance while their F_1 and BC_1F_1 expressed different levels of resistance to TYLCTHV-[2]. This indicated that this *S. habrochaites* accession was a heterozygous plant and its resistance to TYLCV is probably

Table 2. Response of Seedathip3, *S. habrochaites* 'L06112 and their F_1 and BC_1F_1 generation for the presence of TYLCV 45 days after inoculation using ELISA technique. Means with different letters are significantly different at $P \leq 0.05$ according to the Duncan Multiple Range Test.

Phenotypes	ELISA reading with DMRT
04T107 (G)	0.678^{a}
04T104-4	0.608^{ab}
SD3	0.604^{ab}
04T105-2	0.396^{bc}
04T108-5	0.384^{b-d}
04T109-6	0.380^{b-d}
04T104-5	0.334^{c-e}
04T106-4	0.317^{c-e}
04T109-2	0.312^{c-e}
04T106-2	0.302^{c-e}
04T106-3	0.300^{c-e}
04T106-8	0.295^{c-e}
04T107-1	0.295^{c-e}
04T109-9	0.281^{c-e}
04T104-3	0.273^{c-e}
04T107-7	0.271^{c-e}
04T107-5	0.271^{c-e}
04T106-7	0.266^{c-e}
4T105-9	0.258^{c-e}
04T108-6	0.257^{c-e}
04T104-2	0.256^{c-e}
04T109-7	0.241^{c-e}
04T104-7	0.225^{c-e}
04T109-3	0.214^{c-e}
04T106-9	0.214^{c-e}
04T104-1	0.194^{c-e}
04T106 (C)	0.188^{c-e}
04T105-5	0.178^{c-e}
04T109-4	0.123^{c-e}
04T109 (O)	0.112^{c-e}
04T105-10	0.102^{c-e}
04T105-1	0.088^{de}
04T105-7	0.084^{de}
04T105 (B)	0.084^{de}
04T104 (A)	0.073^{e}
04T108 (I)	0.072^{e}
L06112	0.069^{e}

controlled by more than one gene. Resulting BC_1F_1 progenies from this study; 04T105-7, 04T105-1, 04T105-10, 04T109-4 and 04T104-1, showed TYLCV resistance comparable to the *S. habrochaites* 'L60112' parental line. These selected progenies will be useful for breeding programs and for pyramiding genes for resistance to TYLCV. However, resistance was not the only trait that segregated. Several unfavorable characteristics were expressed in the fruit size, color and shape. These character flaws will have to be the focus of further breeding programs in order to develop a more commercially acceptable cultivar that expresses resistance as well as positive fruit quality characteristics.

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