ORIGINAL ARTICLE

Response of resistant breeding lines of tomato germplasm and their progenies with Seedathip3 to *Tomato Yellow Leaf Curl Virus*, Thailand isolate (TYLCTHV-[2])

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Abstract

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Tomato germplasm accessions; FLA456-4, FLA591-15, H24, CLN2443A, CLN2443B, CLN2443C, TLB111, TLB182-1, TLB111-F6-4-1, TLB130-F6-3-1 and TLB134-F6-8-1 from the Asian Vegetable Research Development Center (AVRDC), Taiwan, were screened for resistance to the *Tomato Yellow Leaf Curl Virus*, Thailand isolate (TYLCTHV-[2]). The accessions expressing the resistant genotype were then crossed to the TYLCV-susceptible female parent, Seedathip3 (SD3), to produce F1 hybrids. Tomato parents and their F_1

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progenies were inoculated with TYLCTHV-[2] at 3 weeks of seedling age using viruliferous whitefly (*Bemisia tabaci*) as the inoculation vector. Disease response of the seedling was rated according to the incidence and severity of the development of yellowing and curling symptoms. The presence of TYLCTHV-[2] in the inoculated plants was confirmed by Enzyme-Linked Immunosorbent Assay (ELISA). AVRDC tomato parental lines: H24, FLA591-15 and FLA456-4 expressed mild or no symptoms after one month inoculation. Progeny of crosses between the AVRDC donor parental lines and susceptible Thai cultivars showed intermediate tolerance to TYLCTHV-[2] infection. This indicated that resistance was incompletely dominant.

Key words : geminivirus, *Lycopersicon esculentum*, tomato yellow leaf curl disease, TYLCTHV-[2], breeding for resistance

บทคัดย่อ

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การตอบสนองของมะเขือเทศสายพันธุ์ต่าง ๆกับสายพันธุ์สีดาทิพย์ 3 และลูกผสมที่ได้รับเชื้อใบหงิกเหลืองมะเขือเทศสายพันธุ์ไทย

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โรคใบหงิกเหลืองของมะเขือเทศในประเทศไทยเป็นโรคที่เกิดจากเชื้อ *Tomato Yellow Leaf Curl Virus*, Thailand isolate (TYLCTHV-[2]) แพร่ระบาดโดยมีแมลงหวี่ขาว (*Bermesia tabaci*) เป็นพาหะนำโรค โรคนี้พบในเกือบทุกพื้นที่ที่มีการปลูกมะเขือเทศในประเทศไทย งานวิจัยจึงมุ่งปรับปรุงมะเขือเทศสายพันธุ์ใหม่ให้ต้านทานต่อโรคนี้ โดยศึกษาสายพันธุ์มะเขือเทศจาก ศูนย์วิจัยและพัฒนาพืชผักแห่งเอเชีย (Asian Vegetable Research Development Center, AVRDC) ประเทศไต้หวัน ได้แก่ FLA456-4, FLA591-15, H24, CLN2443A, CLN2443B, CLN2443C, TLB111, TLB182-1, TLB111-F6-4-1, TLB130-F6-3-1 และ TLB134-F6-8-1 และลูกผสมของสายพันธุ์ดังกล่าวกับมะเขือเทศสายพันธุ์สีดาทิพย์ 3 ของไทยที่อ่อนแอต่อโรค โดยใช้ต้นกล้ามะเขือเทศที่มีอายุประมาณ 3 สัปดาห์ มาทดสอบการเกิดโรคโดยถ่ายทอดเชื้อไวรัสด้วยแมลงหวี่ขาวที่ดูดกินอยู่บนต้นมะเขือเทศที่เป็นโรค สังเกตอาการของโรคบนต้นมะเขือเทศที่ได้รับเชื้อทุกสัปดาห์เป็นเวลา 1 เดือน และนำใบอ่อนของมะเขือเทศไปตรวจหาเชื้อไวรัสในสัปดาห์ที่ 2 และ 4 ด้วยวิธี Enzyme-Linked Immunosorbent Assay (ELISA) พบว่า สายพันธุ์พ่อ จำนวน 3 สายพันธุ์คือ H24, FLA591-15 และ FLA456-4 มีความต้านทานต่อโรคในระดับสูง โดยไม่แสดงอาการ หรือแสดงอาการของโรคใบหงิกเหลืองเพียงเล็กน้อยหลังจากได้รับเชื้อเป็นเวลา 1 เดือน ส่วนสายพันธุ์ลูกผสมที่ได้จากการผสมของสายพันธุ์ต้านทานดังกล่าวแต่ละสายพันธุ์กับพันธุ์สีดาทิพย์ 3 พบว่า มีความต้านทานในระดับปานกลางต่อเชื้อ TYLCTHV-[2] แสดงให้เห็นว่าความต้านทานที่ถ่ายทอดมายังลูกผสมเป็นลักษณะเด่นที่ข่มลักษณะด้อยแบบไม่สมบูรณ์

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Tomato yellow leaf curl disease, caused by *Tomato Yellow Leaf Curl Virus* (TYLCV) has become one of the major diseases of cultivated tomatoes (*Lycopersicon esculentum* Mill.) throughout the world. This disease has long been observed and was first described in the Middle East in the 1960s (Cohen and Harpaz, 1964). There also have been several reports of tomato yellow leaf curl disease in Thailand since 1969 in the Central, North Eastern and Northern parts of the country, where tomatoes are cultivated commercially (Sutabutra, 1989).

The most typical symptoms of TYLCV infection are leaf curling and yellowing. TYLCV

can also induce plant stunting and embryo abortion, which adversely affect crop yield and quality. Tomatoes infected with TYLCV at 30 days after planting have shown significant yield loss, and even up to 100% crop loss when plants were infected at earlier growth stages (Nakhla *et al.*, 1994; Green and Kalloo, 1994).

TYLCV is a plant virus, which belongs to the Geminiviridae family and is classified into the genus Begomovirus. The morphology is two joined quasi-isometric particles with circular single-stranded DNA genome(s) (Czosnek and Laterrot, 1997). Most isolates are monopartite which contain only a single genomic component, DNA-A. Some isolates are bipartite and comprised of two isolated components, DNA-A and DNA-B (Navot *et al.*, 1991; Muniyappa *et al.*, 2000).

The TYLCV Thailand isolate can be transmitted either by grafting (Samretwanich *et al.*, 2000) or whitefly (*Bemisia tabaci*). Control of whitefly population is nearly impossible and most of the cultivated tomato cultivars in Thailand are extremely susceptible to TYLCV. Genetically improving tomato resistance to viruses such as TYLCV through breeding, tomato production could be improved to be more sustainable and efficient with less dependence upon insecticides for vector control.

Previously, several *Lycopersicon species* have been discovered and reported to be TYLCV resistant, including *L. peruvianum, L. pimpinellifolium, L. hirsutum* and *L. cheesnmanii* (Scott *et al.*, 1995). However, resistance appears to be controlled by one to five genes depending on the plant source, therefore, several strategies have been used by plant breeders to produce TYLCV-resistant plants incorporating tolerance or resistance gene(s) from the related wild species of tomato into cultivated backgrounds (Vidavsky and Czosnek, 1998).

This research focused on screening and breeding tomato lines with broad spectrum tolerance or resistance to the bipartite form of the tomato yellow leaf curl disease by incorporation of TYLCV resistant gene(s) into cultivated tomato, Seedathip3.

Materials and Methods

Culture of virus, plants and insects:

TYLCTHV-[2] was maintained on susceptible tomato cultivar-Seedathip3 by grafting. Ten plants each of recurrent parent, *L. esculentum* cv. Seedathip3 (SD3) and resistant donor parents to TYLCV-Taiwan: FLA456-4, FLA591-15, H24, CLN2443A, CLN2443B, CLN2443C, TLB111, TLB182-1, TLB111-F6-4-1, TLB130-F6-3-1, TLB134-F6-8-1 and CLN2026D (susceptible check) from the Asian Vegetable Research and Development Center (AVRDC) along with their F_1 progenies, were grown in an insect-proof glasshouse at Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand. The temperature was maintained at 28-30°C. Whiteflies were reared on eggplants in insect-rearing cages outside the glasshouse.

Whitefly-mediated inoculation:

Virus-infected tomato plants were placed with whiteflies in insect-rearing cages and whiteflies were allowed to feed for 72 hr. Three-week-old seedlings of the parents and their F_1 progenies were inoculated with 10-15 viruliferous whiteflies per plant, and caged in screened bottles for another 72 hr. All plants were then transplanted in 12-inch diameter pots and kept in the greenhouse for observation. New shoot initiation after the whitefly inoculation was rated weekly according to the incidence and severity of development of yellowing and curling symptoms using a 0-3 rating scale (Figure 1). Then rating scores were calculated into Disease Severity Index (DSI) values using the formula (Yang *et al.*, 1996):

$$\%DSI = \frac{\Sigma \text{ (Rating Scale x No. of Plants) x 100}}{\text{Total No. of Plants x Highest Rating}}$$

Detection of TYLCTHV-[2]:

TYLCV was quantified and analyzed using the ELISA technique. Each sample consisted of 0.2 g of fresh leaf tissue ground in 0.5 ml of extraction buffer (0.05M Tris-HCl, 0.06M sodium sulphite,

Figure 1. TYLCV symptom rating was determined using a scale of 0-3 where 0 = No symptoms (A), 1= Mild, light yellowing along leaf margins but no curling (B), 2 = Moderate, foliar yellowing and curling (C) and 3 = Severe, leaf curling, puckering and plant stunning (D)

(Color figure can be viewed in the electronic version)

Table 1. Pedigree of tomato genotypes selected by AVRDC as potential sources of resistance to tomato yellow leaf curl disease

Genotype	Origin
FLA456-4	LA 2779 (*L. chilense*, Tyking)
FLA591-15	LA1969 (*L. chilense*, Tyking / Fiona)
H24	*L. hirsutum* f. *glabrarum*
CLN2443A	H24
CLN2443B	H24
CLN2443C	H24
TLB111	H24
TLB182-1	H24 (CLN2114DC$_1$F$_1$-2-29-20-23-14)
TLB111-F6-4-1	H24 (CLN2114DC$_1$F$_1$-2-29-7-2)
TLB130-F6-3-1	H24 (CLN2131DC$_1$F$_1$-96-46-17-6)
TLB134-F6-8-1	H24 (CLN2131DC$_1$F$_1$-96-46-17-32)
CLN2026D	AVRDC / Susceptible check

pH 8.5, Macintosh *et al.*, 1992). Each sample (50 µl/well) was then coated directly on to an ELISA plate and incubated at 37°C for 2 hours. The plate was washed three times with phosphate-buffered saline, containing 0.05% Tween-20 (PBST) for 5 min. between each reagent step. The plate was then blocked with 100 µl per well of 2% BSA in washing buffer to reduce the nonspecific background and incubated for 1 h at 37°C. The plate was then coated with a specific monoclonal antibody (kindly provided by Dr. Oraprapai Gajanandana, National Center for Genetic Engineering and Biotechnology, Thailand) diluted to 1:2000 with PBST. The antibody solution (50 µl) was placed in a well and incubated for 90 min. at 37°C. A goat anti-mouse antibody conjugated with alkaline phosphatase diluted to 1:2000 with PBST was added to each well, and the plate was incubated for another 90 min. at 37°C. Finally, a substrate solution (100 µl of 1 mg/ml of *p*-nitrophenyl phosphate in diethanolamine buffer) was added and the solution was incubated at 37°C for a further 30 minutes. The reaction was stopped by adding 50 µl of 3N NaOH in each well. 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 Analysis

Tomato accessions that were documented as being resistant or tolerant to the TYLCV-Taiwan isolate were used in this study (Table 1). Data were analyzed using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$ for mean separation in SAS program. The tested accessions were divided into two experimental sets in order to separate the effects of environmental differences that were observed in control plants after the inoculation period both in Disease Severity Index (DSI) and ELISA reading. The first set of tested accessions was started 2 weeks prior to the second set. Disease severity of the tested plants was visually observed and scored weekly for 4 weeks, after which the DSI was calculated. Viral accumulation was monitored in the resistant plants of the different accessions by specific monoclonal antisera against TYLCTHV-[2] at 2 and 4 weeks post-inoculation.

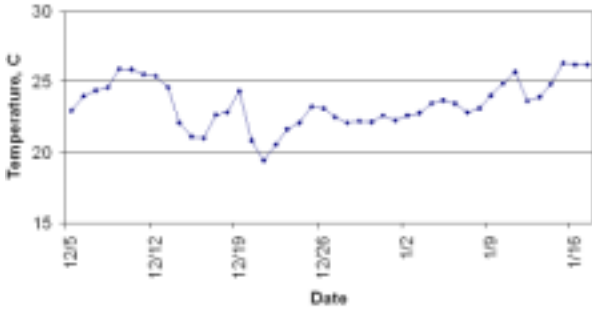

Figure 2. Average temperature at the Tropical Vegetable Research Center (TVRC), Kasetsart University, Kamphaeng Saen during the time of the experimental trial (December 5th to January 16th, 2003).

Results and Discussion

This study was based on classical breeding and a stepwise program approach to develop tomato lines tolerant or resistant to tomato yellow leaf curl disease in Thailand. Various accessions that were documented as being resistant or tolerant to the TYLCV-Taiwan isolate were screened for TYLCV-Thailand isolate.

First set of tested tomatoes were inoculated at the 5th of December, as ambient temperatures increased during that week (average 27/23°C day/night, Figure 2). Susceptible mother, Seedathip3 expressed visual symptoms at the first week after inoculation (Table 2). They showed severe chlorosis and yellowing of the younger leaves and leaf margins. Symptoms developed rapidly and reached a DSI of 74.1% and 100% at 2 weeks and 4 weeks post-inoculation respectively (Figure 3). The second inoculation was initiated on December 19th, and the temperature dropped sharply after inoculation (average 25/20°C day/night). Seedathip3 showed mildly visual symptoms in the second week, although the plants fully developed disease symptoms at 4 weeks post-inoculation (Table 3). This indicated that decreasing temperature after initial inoculation caused a temporary reduction in plant physiological responses which slowed symptom development and viral replication.

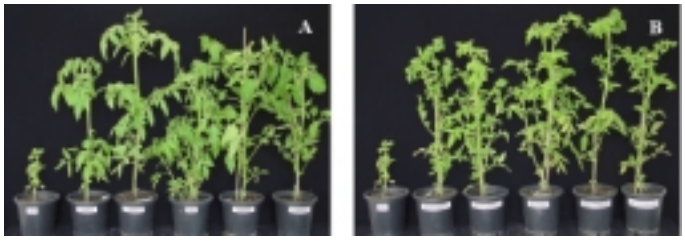

Figure 3. TYLCTHV-[2] symptoms at 4 weeks post inoculation: (A) parental lines: SD3, FLA456-4, FLA591-15, H24, CLN2443A, CLN2443B and (B) SD3 and F_1 progenies crosses with FLA456-4, FLA591-15, H24, CLN2443A, CLN2443B

(Color figure can be viewed in the electronic version)

Table 2. Disease Severity Index (DSI, %) and ELISA readings at 2 and 4 weeks post-inoculation with TYLCTHV-[2].

Cultivars	2 weeks		4 weeks	
	DSI (%)	ELISA reading	DSI (%)	ELISA reading
FLA456-4	12.5	0.042^{f}	33.3	0.150^{ef}
SD3X FLA456-4	55.6	0.452^{bcd}	70.4	0.455^{bcd}
FLA591-15	0	0.007^{f}	33.3	0.016^{f}
SD3X FLA591-15	63.3	0.502^{bcd}	86.7	0.303^{cde}
H24	0	0.012^{f}	3.3	0.014^{f}
SD3 X H24	55.6	0.246^{def}	63	0.402^{bcde}
CLN2443A	0	0.002^{f}	0	0.448^{bcd}
SD3 X CLN2443A	60	0.350^{cde}	70	0.672^{ab}
CLN2443B	0	0.145^{ef}	27.8	0.276^{def}
SD3 X CLN2443B	60	0.389^{bcde}	73.3	0.579^{abc}
CLN2026D	66.7	0.847^{a}	100	0.784^{a}
SD3 X CLN2026D	88.9	0.613^{abc}	100	0.813^{a}
SD3	74.1	0.640^{ab}	100	0.750^{a}

Means with different letters within ELISA reading dates are significantly different at P≤0.05 according to the Duncan Multiple Range Test.

Table 3. Disease Severity Index (DSI, %) and ELISA readings at 2 and 4 weeks post- inoculation with TYLCTHV-[2].

Cultivars	2 weeks		4 weeks	
	DSI (%)	ELISA reading	DSI (%)	ELISA reading
TLB111	6.7	0.000^{d}	10	0.052^{e}
SD3 X TLB111	20	0.167^{c}	70	0.312^{d}
TLB182-1	0	0.007^{d}	16.7	0.140^{de}
SD3 X TLB182-1	10	0.241^{bc}	66.7	0.551^{c}
TLB111-F6-4-1	0	0.000^{d}	0	0.047^{e}
SD3 X TLB111-F6-4-1	10	0.232^{c}	63.3	0.309^{d}
TLB130-F6-3-1	0	0.009^{d}	0	0.045^{e}
SD3 X TLB130-F6-3-1	3.3	0.011^{d}	0	0.221^{de}
TLB134-F6-8-1	36.7	0.290^{bc}	100	0.813^{ab}
SD3 X TLB134-F6-8-1	43.3	0.356^{ab}	100	0.651^{bc}
CLN2443C	6.7	0.006^{d}	0	0.041^{e}
SD3 X CLN2443C	6.7	0.044^{d}	20	0.289^{d}
SD3	43.3	0.427^{a}	100	0.889^{a}

Means with different letters within ELISA reading dates are significantly different at P≤0.05 according to the Duncan Multiple Range Test.

The donor parents of several accessions developed no or mild symptoms of tomato yellow leaf curl disease after one month of inoculation, but when a recurrent susceptible line, "Seedathip3", was crossed with these resistant donors to advance the breeding generation, disease severities of F_1 progenies were intermediate in their response to TYLCTHV-[2]. This indicates that resistance, at

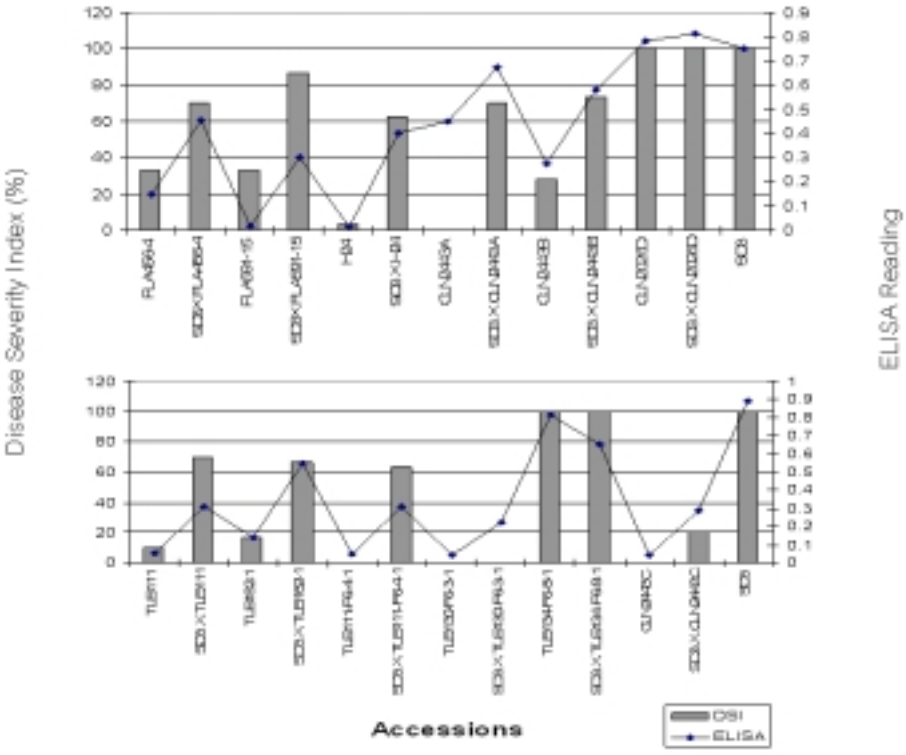

Figure 4. Comparison of visual observations with serological indexing of TYLCTHV-[2] at 4 weeks post-inoculation.

least in these plants, is incompletely dominant. It also showed that the donor parents possessed a high level of tolerance to TYLCTHV-[2] but were not immune.

L. hirsutum has been reported to be symptomless to TYLCV infection. This resistance is apparently controlled by one dominant major gene in wild species accessions LA1777 and LA 368 (Vidavsky and Czosnek, 1998). In 1990, Kalloo and Benerjee developed H24 from *L. hirsutum* f. *glabrarum* as a source of resistance to TYLCV. H24 has shown resistance to both the TYLCV at the AVRDC, Taiwan and to ToLCV in Bangalore, India. The resistance in H24 was isolated as a single gene named *Ty-2* and has been mapped as an introgression, which is located on the lower end of chromosome 11 between markers TG36 and TG393 (Hanson *et al.*, 2000). In this study, H24 also exhibited the best resistance performance to TYLCTHV- [2]; however H24 derivatives showed varying reactions to the TYLCTHV- [2], with symptoms varying from none to severe. TLB111 and TLB182 displayed similar responses to TYCLV inoculation. Both cultivars were derived from the same accession, CLN2114, crossed with different lines, DC1F1-2-29-7-2 and DC1F1-2-29-20-23-14. TLB134-F6-8-1, which came from accession CLN 2131DC1F1-96-46-17-32, exhibited severe virus symptoms in the third week and disease development reached 100% after one month. Accession TLB130-F6-3-1, which also came from CLN2131 DC1F1-96-46-17-6, a different line, was asymptomatic and had a low ELISA reading of TYLCTHV-[2] concentration.

Resistance to TYLCV in *L. chilense* is controlled by the major gene *Ty-1*, which exhibits a reduction of the virus titers and long-distance movement of the virus in the plant (Michelson *et al.*, 1994). *Ty-1* was mapped on chromosome 6 using RFLP markers (Zamir *et al.*, 1994). Both

FLA591-15 (LA1969, Tyking/Fiona) and FLA 456-4 (LA2779, Tyking) used *L. chilense* as the resistance donor; however, in this study Tyking/ Fiona displayed a higher resistance to TYLCTHV-[2] than Tyking alone.

Comparison between ELISA detection and %DSI indicated differences among the readings of susceptible, resistant and tolerant accessions (Figure 4). Resistant plants accumulated considerably less virions than susceptible lines. Values were dramatically increased in susceptible genotypes, which showed both severe symptoms and high readings, but CLN2443A, which had a low percentage of DSI, showed a relatively high virus titer, indicating tolerance.

The viral concentration of the tomato plants tested was low at the second week after inoculation, but increased over time. However, positive control CLN2026, a highly susceptible line had slightly less viral titer at the 4th week than at the 2nd week because of plant weakness. Plants dramatically deteriorated after the second week and some of them died during the experiment, resulting in the reduction in viral accumulation of TYLCV in the plants. This suggests that time of detection is one of the key factors to consider for a determination of the resistance level.

Conclusion

This study has shown that some of the tomato accessions with TYLCV-Taiwan resistance are a good genetic source for resistance or tolerance to TYLCTHV-[2], and those accessions could be useful for the control of TYLCV in Thailand by effectively reducing the reliance on insecticides. The response to TYLCV would need to be assessed in detail to confirm the stability of resistance, while selecting resistant types that have agronomic characteristics suitable for local conditions and market potential for use in local breeding programs.

Acknowledgement

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