Original Article

Resistance to tomato yellow leaf curl virus -Thailand isolate (TYLCTHV-[2]) and markers loci association in BC$_2$F$_1$ population from a cross between Seedathip 3 and a wild tomato, Solanum habrochaites ‘L06112’ clone no.1

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Abstract

The BC$_2$F$_1$ population from a cross between wild tomato, Solanum habrochaites ‘L06112’ and recurrent susceptible variety, Seedathip 3 was investigated for a resistance to Tomato yellow leaf curl Thailand virus (TYLCTHV-[2]). Five stem cuttings from each of the 196 lines were inoculated with TYLCTHV-[2] using viruliferous whiteflies as the inoculation vector. Disease response was recorded weekly intervals and scored for three weeks according to the severity of the symptoms. The presence of TYLCTHV-[2] was confirmed by enzyme-linked immunosorbent assay (ELISA) at three weeks after inoculation. ELISA readings showed a normal distribution for the BC$_2$F$_1$ population ranging from 0.055 (resistant) to 0.930 (susceptible). DNA samples from BC$_2$F$_1$ population were analyzed for genes and markers association; Ty-2 on chromosome 11 using AVRDC primer number 11-090.0 (TG105A), and Ty-3 on chromosome 6 using marker C2_At3g11210. Results showed that Ty-2 was lost during stepwise selection at BC$_1$F$_1$ generation, while Ty-3 showed no relationship to marker C2_At3g11210 and the amount of virus detected from ELISA reading. This indicated that the TYLCTHV-[2] resistant phenotype response in the BC$_2$F$_1$ population neither came from Ty-2 nor linked to the Ty-3 gene.

Keywords: breeding, disease resistance, TYLCV, TYLCTHV-[2], wild tomato introgression, Solanum habrochaites

1. Introduction

Tomato yellow leaf curl disease is transmitted by whiteflies (Bemisia tabaci), and it is caused by the tomato yellow leaf curl virus (TYLCV). TYLCV is a circular single-stranded DNA with quasi-isomeric particles. Most isolates found are monopartite, while the Thailand isolate (TYLCTHV-[2]) is comprised of two genomes; DNA-A and DNA-B and hence named bipartite. The obvious symptoms are systemic in expression, starting with chlorotic curling of the youngest leaves. Plants infected at an early growth stages show stunting soon after infection and are incapable of producing any kind of quality crop.

The disease can be managed by cultural practices, selecting and planting resistant species to the virus or by managing the whitefly population through insecticides. Since
frequent insecticide applications are resource costly, both in time and money, breeding for resistance is a desirable approach in order to rectify this serious problem. Introgression of TYLCV resistant genes from wild to commercial species has been done for decades with several species of **Solanum; S. peruvianum, S. pimpinellifolium, S. habrochaites and S. cheesmaniae** (Scott et al., 1995), and in most cases, multiple responsible genes (1 to 5 genes, either recessive or dominant resistance) were found in each species depending upon its geographic origin (Vidavsky and Czosnek, 1998).

Several accessions from **S. habrochaites** were used as donor parents for a source of TYLCV resistance. Hassan et al. (1984) found **S. habrochaites** accession ‘LA386’ was TYLCV-resistant in Jordan, while Ioannou (1985) found that ‘LA1777’ resistant to TYLCV in Cyprus. In 1998 Vidavsky and Czosnek developed a resistant line by combining ‘LA386’ and ‘LA1777’ and found that their new variety resisted TYLCV in Israel.

Previous reports on H24, the cross between **S. habrochaites** f. **glabrarum** accession ‘B6013’ and **S. lycopersicum** ‘Hisar Arun’ followed by 4 backcrossing and 2 generations for self-crossing, showed high levels of resistance to viral isolates from Taiwan (TYLCV-TW), and tolerance to TYLCV-SL from Sri Lanka and TYLCV-Ban3 from India (Zamir et al., 1994). A genetic study for molecular markers revealed the gene responsible for TYLCV resistance was located on chromosome 11 between RFLP markers TG36 and TG393 and named Ty-2 (Hanson et al., 2000).

In 2007, H24 and several other tomato germplasm were crossed with the Thailand commercial susceptible cultivar, Seedathip3. Parents with their F₁ hybrid were infected with Thailand’s viral isolate (TYLCTHV-[2]) and they showed the highest level of resistance with no symptoms to TYLCTHV-[2] in H24, while F₁ progenies showed a moderate resistance (Chomdej et al., 2007).

Because most of Thai commercial cultivars are susceptible to TYLCV, this study has been based mainly on exploiting traits from the wild tomato, **Solanum habrochaites** accession no.L06112 clone no.1 (L06112-C1) into a domestic cultivar, Seedathip3. Screening for resistance and studying the distribution in BC₁ of tomato population was validated using enzyme-linked immunosorbent assay (ELISA). In addition, phenotypes for disease response were analyzed for the distribution in BC₁, BC₂, F₁, L06112, and Seedathip3 were caged together with viruliferous whiteflies. Shoots of infected plants were scored at weekly intervals for three weeks starting at the first week after inoculation according to disease incidence and disease severity (Figure 1).

2.2 Plant inoculums and whitefly cultures

A susceptible tomato cultivar, Seedathip3, was used as a source of TYLCTHV-[2] culture and inoculums were propagated by cleft grafting from the infected plants to one month old seedlings in a glasshouse. Infected plants developed full symptoms in two weeks after being grafted. Whiteflies were reared on eggplants in a separate greenhouse to avoid contamination.

2.3 Whitefly-mediated inoculation

Whiteflies were subjected to inoculated plants and allowed to feed for a week, and then five cuttings of tested plants (BC₁, BC₂, F₁, L06112, and Seedathip3) were caged together with viruliferous whiteflies. Shoots of infected plants were scored at weekly intervals for three weeks starting at the first week after inoculation according to disease incidence and disease severity (Figure 1).

2.4 Detection of TYLCTHV-[2]

Viral accumulation was quantified from infected plants three weeks after inoculation using ELISA technique (Gajanandana et al., 2002). New shoots from each tested plant were ground in extraction buffer in the ratio of 1:250 g/ml and coated to the 96-well ELISA plate. The plate was incubated

Figure 1. TYLCV symptom rating was determined using scale of 0 to 3 where 0 = No symptoms (A), 1 = Mild, light yellowing along the leaf margins but no curling (B), 2 = Moderate, foliar yellowing and curling (C) and 3 = Severe, leaf curling, puckering and plant stunning (D)
at 37°C for two hours and washed three times at 5 min intervals with phosphate buffer, containing 0.05% tween-20 (PBST). The plate was blocked with 2%BSA in PBST and then incubated for one hour at 37°C. After washed three times with PBST, a specific monoclonal antibody was added to each well and the plate was incubated at 37°C for 90 min. The plate was washed again for three times at 5 min. intervals and a goat anti-mouse antibody conjugated with alkaline phosphate was added to each well and then incubated for another 90 min at 37°C. A substrate solution containing p-nitrophenyl phosphate in diethanolamine buffer was added to each well and incubated at 37°C around 30 min for color reaction. The reaction was stopped by adding 3N NaOH in each well and was measured as absorbance (optical density = OD at 405 nm on an ELISA plate reader, Multiskan EX, Themosystems OY, Finland). Tested plants were evaluated for infection by comparing to a positive threshold value (PTV). Plants that had an ELISA reading three times higher than a negative control was considered as infected (Sutara et al., 1986).

2.5 Markers linkage analysis

The DNA of 196 lines from BC$_2$F$_1$ population was extracted according to the methods described by Fulton et al. (1995). Samples were amplified by polymerase chain reaction (PCR) technique with forward and reverse specific primers; (TG105A for Ty-2 and C2_At3g11210 for Ty-3) linked to resistant allele at loci on chromosome 11 and 6, respectively (Hanson, personal communication; Garcia and Maxwell 2007; Ji et al., 2007; Sol Genomic Network). Amplification conditions started at 35 cycles of 2 min at 94°C DNA denaturation, 30 sec at 50°C annealing for marker TG105A and 60°C annealing for marker C2_At3g11210. Then the extension period was 1 min at 72°C for both markers. After that amplified DNA products were cut with restriction enzymes, *taqI* for marker TG105A at 65°C for 3-4 hours and *Hinf*I for marker C2_At3g11210 at 37°C for 3-4 hours incubation period. Then the digested fragments were separated in 2% agarose gels. Linkage between markers and phenotypes for TYLCHV-[2] response were analyzed on BC$_2$F$_1$ population by MapQTL® version 4.0 with the Kruskal-Wallis one–way analysis of variance (van Ooijen et al., 2002).

3. Results and Discussion

Even though wild species contain numerous sources of resistances for both insects and diseases (Rick and Chetelat, 1995), gene complexity and linkage drag could be a major obstacle factor for plant breeders. Because the accession no. L06112 from *Solanum habrochaites* and Seedathip3 from *S. lycopersicum* are not closely related, interspecific hybridization only occurred when using Seedathip3 as a female parent (Whankaew et al., 2005). Moreover, the F$_1$ hybrid still showed paternal self-incompatibility and did not bear fruit at the F$_2$ generation. Therefore plants at the F$_2$ generation were backcrossed to the recurrent parent (Chomdej and Chunwongse, 2006; Chomdej et al., 2007).

In previous research, the BC$_2$F$_1$ generation was screened for TYLCHV-[2] resistance and the results showed the top five performance lines were A-1, B-1, B-7, B-10, and O-4, which contained low viral concentrations asymptomatic to mild symptoms in infected tested plants. Fruits from selfing in BC$_2$F$_1$ generation were varied in size, shape and color; therefore breeding line no. B-7, which acquired closer characteristics to their recurrent parent, Seedathip3, has been selected for advancements into a further breeding program. Unfortunately, seeds derived from selfing BC$_2$F$_1$ generation exhibited very low germination rates, which was less than 50% of the best rate of germination (unpublished data). Consequently another attempt at backcrossing has been proceeded (Chomdej et al., 2008) (Figure 2).

Figure 2. Diagram of a breeding plan; *Solanum habrochaites* accession no. L06112 was crossed to *S. lycopersicum* var. Seedathip3 to produce F$_1$ generation. The resistant hybrids were backcrossed to a susceptible Seedathip3 to generate BC$_1$F$_2$ and BC$_2$F$_1$ generation.
The disease severity and viral titer in susceptible lines showed a high correlation by ELISA detection. Plants containing high values of TYLCTHV-[2] accumulation, also showed moderate to severe disease symptoms (score ≥ 2). Only ten out of 196 plants had ELISA reading values lower than the PTV value (0.260) and the average symptom scoring were ranging from 1.4 to 2.4.

ELISA readings showed a normal distribution for the BC$_F$$_1$ population ranging from 0.055 (resistant) to 0.930 (susceptible) (Figure 3). This indicated that the resistance response to TYLCTHV-[2] from ‘L06112’ is quantitatively inherited and controlled by multiple genes. Several reports have shown similar results that wild tomatoes, which were crossed to introgress genes for resistance, appeared to be controlled by more than one gene depending on plant source and background (Vidavsky and Czosnek, 1998; Hanson et al., 2000; Ilana et al., 2009; Ji et al., 2009).

Tomato commercial line, H24 was analyzed for a resistant gene Ty-2 from a cross to a wild species, S. habrochaites accession B6013. This resistance was mapped to the long arm region of chromosome 11 between marker TG393 and TG36 (Kaloo and Banerjee, 1990; Hanson et al., 2000). AVRDC primer number 11-090.0 (Hanson, personal communication), TG36 (Kaloo and Banerjee, 1990; Hanson et al., 2000). A VRDC gene in our population. The marker linked to TYLCTHV-[2] resistance gene. Results from a Chi-square test showed a segregating population in the marker C2_At3g11210 detection in the 196 tested lines from BC$_F$$_1$ with a segregation ratio of 1:1 (102:94 lines). In addition, the ELISA mean values of 102 plants that were detected by the marker C2_At3g11210 and 94 plants that did not link to the marker were 0.531088 and 0.502606, respectively. These numbers showed no correlation between Ty-3 marker and tomato lines that expressed resistance at a statistical significance (p<0.05) based on the Kruskal-Wallis one-way analysis of variance in MapQTL® 4.0.

Further confirming in detection of Ty-3 marker showed no association to resistance by comparing two groups of DNA samples (extremely resistant and susceptible) in the BC$_F$$_2$ population. Nucleotide polymorphism was found between parents’ designated L06112 carrying Ty-3 gene, which resisted to monopartite TYLCV and Tomato mottle virus (ToMoV) in genus begomovirus. Tested tomatoes that showed low levels of viral concentration at BC$_F$$_1$ generation (BC$_F$$_2$–resistant) were detected for Ty-3 marker at the ratio of 7/10 plants, while high levels of viral titer (BC$_F$$_1$–susceptible) showed 6/10 plants (Figure 5). It indicated that Ty-3 was not responsible for the TYLCTHV-[2] resistance. However, this gene appears to be useful practically for broad spectrum improvement purposes and would be incorporated into our breeding program. In the ongoing research genome scan using more markers is underway to localize QTLs controlling TYLCTHV-[2] resistance.

![Figure 3](image3.png) ELISA readings showed a normal distribution of response to TYLCTHV-[2] resistance in BC$_F$$_1$ population from a cross between Seadathip3 and a wild tomato, Solanum habrochaites ‘L06112’ clone no.1 at three weeks post inoculation.

![Figure 4](image4.png) PCR fragments with the Ty-2 locus primers (TG105A) Lane 1: 100-bp marker (Promega Corp.); Lane 2: Seadathip3 (susceptible), Lane 3: L06112-C1 (resistant to TYLCTHV-[2]); Lane 4: line B (F$_1$, resistant to TYLCTHV-[2]); Lane 5: line B-7 (BC$_F$$_1$, resistant to TYLCTHV-[2]); Lane 6-15: BC$_F$$_1$ population (extremely resistant to TYLCTHV-[2]); Lane 16-25: BC$_F$$_2$ population (extremely susceptible to TYLCTHV-[2]).
4. Conclusion

In conclusion, progress in breeding traits from wild species is a slow process, primarily because of the complex genetics of the plants. Multiple genes are involved in the resistance, but TYLCTHV-[2] resistance found in our BC$_{F_1}$ population derived from a cross between *Solanum habrochaites* ‘L06112’ clone no.1 and Seedathip3 was not affected by the Ty-2 and Ty-3 gene. However, both genes will be a great benefit for further breeding programs attempting to broaden the base of resistance for TYLCV around the world. From this study we believe that TYLCTHV-[2] resistance was quantitatively inherited from donor parents and controlled by a concert of multiple genes, either major or minor.

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References


