Protocol for Ty-1 Gene Detection (Tomato Yellow Leaf Curl Virus Resistance) in Tomatoes

Description

Tomato yellow leaf curl virus (TYLCV) is the name given to a complex of geminiviruses infecting tomato cultures worldwide. TYLCV is transmitted by a single insect species, the whitefly *Bemisia tabaci*. (Murad Ghanim and Henryk Czosnek, 2000)

Diseases caused by Whitefly-transmitted geminiviruses are major constraints in tropical and subtropical tomato production. Geminiviruses are highly variable and include monopartite and bipartite forms. High levels of resistance have been identified in wild relatives of tomato such as *Lycopersicon chilense, L. hirsutum, L.pimpinelliforium,L.cheesmanii,* and *L. peruvianum.* Some of these resistance genes were transferred and utilized in cultivated tomato,including *Ty-1* from *L.chilense* (Zamir *et. al.,* 1994) and *Ty-2* from *L. hirsutum* (Kalloo and Banerjee, 1990; Hanson *et. al.,* 2000). Both *Ty-1* and *Ty-2* have been mapped to chromosomes 6 and 11, respectively. Resistance conferred by these genes independently provides protection to some,but not all, stains of Tomato Yellow Leaf Curl Virus (TYLCV). Nevertheless, both *Ty-1* and *Ty-2* will be major components in developing varieties with multigenic resistance to geminiviruses.

Description

Whitefly transmitted geminiviruses in tomato are among the most destructive of all viruses in tomato. There are perhaps over 100 reported whitefly transmitted geminiviruses in tomato and all tend to be called either tomato yellow leaf curl virus (TYLCV) or tomato leaf curl virus (TLCV). The differences between the two groups tend to be variations in the genome with TYLCV having a bipartite genome while TLCV having a monopartite genome. However, this is somewhat confusing as there are major differences between many of the bipartite TYLC viruses, genome as well as host range, severity, geographical location, as too the TLC viruses. Further, some monopartite viruses have been called TYLCV and some bipartite viruses, TLCV. Grouping of the viruses has therefore tended to relate to their resistance source in tomato and geographical region.

Resistance

High levels of resistance to the tomato yellow leaf curl viruses have been identified in wild relatives of tomato including *Lycopersicon hirsutum*. In total five geminivirus resistance loci have been mapped, two on chromosome 11 and three on chromosome 6. Several resistance genes, including *Ty-1* (chromosome 6), *Ty-2* and *Ty-3* (chromosome 6) have been transferred to table and processing type tomatoes. *Ty-1* has been transferred from LA1969 (*L.chilense*) to a number of common cultivated tomatoes.

Ty-2 has been transferred to the cultivar “H24” from *L. hirustum*. “H24” confers resistance to a very, very narrow range of TYLCV strains which are only found in Taiwan, Israel, and some parts of northern Vietnam and southern parts of India. As these are not the major tomato growing areas of Asia, the application of *Ty-2* resistance for Chia Tai is extremely limited, but nonetheless good to

know and have available in breeding lines. **What is important is that stacking the three known genes, *Ty-1*, *Ty-2* and *Ty-3* confers better tolerance to most TYLCV and TLCV isolates than *Ty-3* or a combination of two.**

The Ty-1 gene

The Ty-1 gene has been linked to chromosome 6. The resistance mechanism associated with this gene is currently not clear. What is clear is that the resistance conferred by this gene is limited. It also appears that the resistance is not "true resistance', rather it is tolerance. The resistance **is incomplete dominance from a single major gene (Ty-1) and two or more modifier genes**. The Ty-1 region on chromosome 6, in the region around markers TG297 and TG97, is very tightly linked to the Mi-gene which confers resistance to nematodes. **So in undertaking screening for Ty-1 resistance, the presence of the Mi-gene should also be confirmed.**

Breeding

The below protocol detects the *Ty-1* gene locus using a marker. For breeding purposes **resistance is incomplete dominance with additional modifier genes** so **BOTH parents need to be "resistant" for the F1 to also be resistant**. Time taken for the detection is approximately one day. It is more efficient, time-wise, to do up to 93 samples at a time, this being the number of wells in the microtitre plate used for the extraction as well as the number of wells in the PCR machine. Doing ten samples takes nearly as long as 93 samples!! If both parents are resistant, there should be no need to confirm the status of their progeny.

Protocol

1. Tomato leaf DNA extraction

Extract tomato leaf (any stage) DNA using a modified NaOH lysis procedure.

1.1 Label each plant.

1.2 Cut a small "disc" of tomato leaf tissue using a 1.5 ml eppendorf tube.

1.3 Place the leaf disc into a 96 well plate or 1.5 ml eppendorf tube as a pre-designated.

1.4 Add 50 µl of 0.5 M NaOH into each well *(0.5 M NaOH: 2 g NaOH per 100 ml of dH_2O)*.

1.5 Grind leaf tissue to a liquid consistency using a pestle. Optionally, a pestle in a small bench-top hand drill can be used.

1.6 After each sample is ground, wash the pestle in a 0.1% chlorine (final) solution and rinse three times with R/O.

1.7 Precipitate the leaf extracts by centrifuging in a microtiter plate benchtop centrifuge at 200 rpm, 4°C for 10 minutes or if using eppendorf tubes, at maximum speed (~13,500 rpm) in a microfuge for 2 minutes.

1.8 Transfer 2 µl of plant extract to a 96 well plate (or eppendorf tube) containing 198 µl of 0.1 M Tris-HCl, pH 8.0 (1:100 dilution). Mix by pipetting (eppendorf tube by vortexing).

1.9 Centrifuge at 200 rpm, 4°C for 10 minutes or if using eppendorf tubes, at maximum speed (~13,500 rpm) in a microfuge for 2 minutes.

1.10 Store at –20°C or 4°C overnight.

Controls:

Homozygous Resistance	– None available yet (H24 is not Ty-1)
Heterozygus Resistance	–
Susceptable	– 947 (CT)

2. PCR

Primers*
Primer JB-1F: AAC CAT TAT CCG GTT CAC TC
Primer JB-1R: TTT CCA TTC CTT CTT TCT CTG

Preparation of PCR master mix: as follows:

Reagents	10 μl Reaction	Master mix (97 reactions) of 10 μl each
10 mM dNTPs	0.5	48.5
10X Buffer	1.0	97
50 mM $MgCl_2$	0.25	24.25
Primer JB-1F (10 pmole/μl)	0.5	48.5
Primer JB-1R (10 pmole/μl)	0.5	48.5
dH_2O	6.15	596.55
Taq DNA Polymerase** (5 u/μl)	0.1	9.7
Total	9	873
DNA template	1	-
Total volume	10	-

- Pipette 9 μl of PCR master mix to PCR tubes.
- Add 1 μl of DNA template.

NB: Don't forget the PCR Negative control

* Ana Perez de et.al., 2007. Identification of a CAPS marker tightly linked to the Tomato yellow leaf curl disease resistance gene *Ty-1* in tomato. European Journal of Plant Patholology. 111:347-356.

** Check the stock *Taq* DNA polymerase concentration first

PCR reaction:

PCR reaction performed in a PCR machine using the following profile:

1 cycle:	94°C for 3 minutes
30 cycles:	94°C for 30 seconds
	55°C for 30 seconds
	72°C for 30 seconds
1 cycle:	72°C for 10 minutes

3. Restriction enzyme digestion of PCR amplified products

Digest PCR products with *TaqI* restriction enzyme.

TaqI restriction enzyme site:

T/CGA
AGC↓T

Preparation of restriction digestion master mix: as follow:

Reagents	Single Reaction (µl)	Master mix (50 reactions) of 2 µl each
Taq I (20 U/ µl)	0.1	5
NE Buffer 3	1.2	60
dH_2O	0.7	35
Total	2	100
PCR Product	10	-
Total	12	

Incubate at 65 º C for 3 hours by PCR machine.

4. Agarose Gel Electrophoresis

Electrophoresis DNA in 1.5 % agarose gels at 100 volt for approximately 30 minutes (this will vary depending on the electrophoresis unit used).

- Prepare 1.5 % agarose gel in 0.5X TBE buffer and dissolve by heating in a microwave.
- Cool the molten agarose to 50 - 60º C, pour into the mold, insert the comb and allow the gel to set
- Submerge the gel in 0.5X TBE buffer and remove the comb.
- Load the DNA approximately 10 µl into the wells. Include the appropriate molecular weight marker as weel as the negative and positive controls.
- Run the gel at 100 volts for approximately 30 minutes until the bromophenol blue dye front is within 1 cm of the end of the gel.

5. Visualisation of DNA using SyberGold

- Submerge the gel and stain in SyberGold Solution (5µl SyberGold in 50ml 0.5X TBE Buffer) for 15 minutes.
- Visualize the restricted amplified DNA fragments under ultraviolet-illumination (Gel Documentation).
- Verify results against DNA molecular weight and positive, negative control.

6. Analysis of DNA fragments pattern

- Verify results against DNA molecular weight markers (MW) and the positive and negative controls. This indicates the absence or presence of the loci (Figure 1).

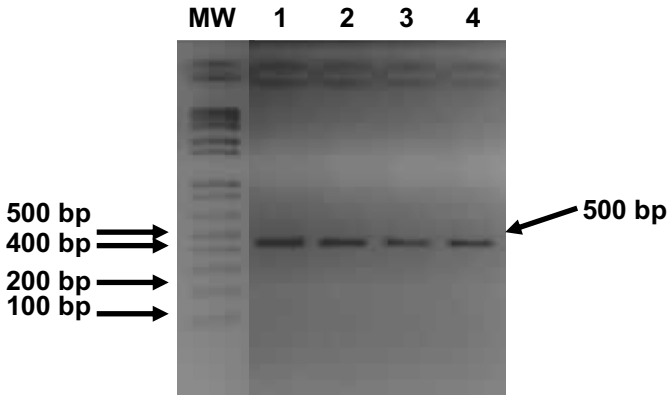

Figure 1. Agarose gel electrophoresis of PCR amplified DNA associated Ty-1 gene detection in Tomato. Lanes 1 – 4 are susceptible. No homozygous or heterozygous lines are available at this time.

7. Summary

The above result is only indicative of susceptible lines as no homozygous nor heterozygous lines were available at the time of writing this protocol. LA3473 and LA1969 are both homozygous for Ty-1 but no seeds are available at this stage. Below is a summary of resistances taken from the above reference (Ana Perez de *et.al*., 2007).

Table 1 Plant material analysed

Species	Accession	*Mi*[a]	*Ty-1*[b]	Aps-1[c]	REX-1[d]	JB-1[e]	Source[f]
S. lycopersicum	UPV21183	*mi/mi*	*ty-1/ty-1*	1	1	1	1
	UPV21745	*mi/mi*	*ty-1/ty-1*	1	1	1	1
	FC	*mi/mi*	*ty-1/ty-1*	1	1	1	1
	Gévora	*Mi/Mi*	*ty-1/ty-1*	1	2	1	2
	H1124	*Mi/Mi*	*ty-1/ty-1*	2	2	2	2
	Fitó 1	*Mi/Mi*	*ty-1/ty-1*	1	2	1	3
	Fitó 2	*Mi/Mi*	*ty-1/ty-1*	2	2	2	3
	Fitó 3	*mi/mi*	*Ty-1/Ty-1*	2	1	3	3
	Fitó 4	*Mi/Mi*	*Ty-1/Ty-1*	2	2	3	3
	SC	*mi/mi*	*Ty-1/Ty-1*	2	2	3	4
	Boludo	*Mi/mi*	*Ty-1/ty-1*	1/2[g]	1/2	3	5
	Anastasia	*Mi/mi*	*Ty-1/ty-1*	1/2	2	3	5
	TY197	*mi/mi*	*ty-1/ty-1*	1	1-2[h]	1	6
	LA3473	*mi/mi*	*Ty-1/Ty-1*	2	3	3	7
	UPV21008[i]	*mi/mi*	*ty-1/ty-1*	1	1	1	1
S. peruvianum	PI128657	*Mi/Mi*	*ty-1/ty-1*	2	2-2/3	3	8
	UPV20196	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20340	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20342	*mi/mi*	*ty-1/ty-1*	2	3	3	1
	UPV20345	*mi/mi*	*ty-1/ty-1*	2	2	3	1
S. chilense	LA1969	*mi/mi*	*Ty-1/Ty-1*	2	2	3	7
	LA2884	*mi/mi*	*ty-1/ty-1*	2	2	3	7
	UPV20304	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20306	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20310	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20320	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20328	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20329	*mi/mi*	*ty-1/ty-1*	2	2	3	1
	UPV20336	*mi/mi*	*ty-1/ty-1*	2	2	3	1
S. habrochaites	LA0386	*mi/mi*	*ty-1/ty-1*	3	2	D_1^j	7
	LA1777	*mi/mi*	*ty-1/ty-1*	3	2	D_2	7
	UPV16910a	*mi/mi*	*ty-1/ty-1*	2	2	D_3	1
	UPV17046 E	*mi/mi*	*ty-1/ty-1*	2	2	D_4	1
S. pimpinellifolium	LA1636	*mi/mi*	*ty-1/ty-1*	2	1	2	7
	LA1670	*mi/mi*	*ty-1/ty-1*	2	1	2	7
	LA2182	*mi/mi*	*ty-1/ty-1*	2	1	1	7
	LA2188	*mi/mi*	*ty-1/ty-1*	2	1	1	7
	LA2725	*mi/mi*	*ty-1/ty-1*	2	1	2	7
	PI 390728	*mi/mi*	*ty-1/ty-1*	2	1	1	9
	PI 127807	*mi/mi*	*ty-1/ty-1*	2	1	2	9

[a] Alleles for the *Mi* gene: *M*i resistant allele, *mi* susceptible allele

[b] Alleles for the *Ty-1* gene: *Ty-1* resistant allele, *ty-1* susceptible allele

[c] Alleles for the Aps-1 marker (see results for description)

[d] Alleles for the REX-1 marker (see results for description)

[e] Allele for the JB-1 marker (see results for description)

[f] Source: 1: Genebank of the Institute for the Conservation and Improvement of Agrodiversity (COMAV), Valencia, Spain; 2: J. Gragera, Sevicio de Investigación y Desarrollo Tecnológico, (SIDT) Badajoz, Spain; 3: Semillas Fitó S.A., Barcelona, Spain; 4: Plant material with this genetic composition belongs to a seed company; 5: Seminis Vegetable Seeds, Murcia, Spain; 6: Dr. M. Pilowsky, Volcani Center, Rehovot, Israel; 7: Tomato Genetics Resource Center (TGRC), University of California, Davis; 8: United States Department of Agriculture (USDA); 9: Australian Plant Genetic Resource Information Service (AusPGRIS), corresponding genebank codes are AUSTRCF311996 (PI 390728) and AUSTRCF312128 (PI127807)

[g] Bars separate alleles present in heterozygous individuals

[h] Hyphens separate different patterns for different individuals of a concrete plant material

[i] This accession was formerly classified as *L. esculentum* var. *cerasiforme*

[j] D: Alleles different than the ones described for the rest of the species

Trouble Shooting