Detection of *Ty-2* Gene (Tomato Yellow Leaf Curl Virus Resistance) in Tomato

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-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-2 gene

The Ty-2 gene has been linked to chromosome 11 (see Figure 1). The resistance mechanism associated with this gene is currently not clear. What is clear is that the resistance conferred by this gene is, as mentioned above, limited to only a few TYLCV isolates.

While the *Ty-2* gene confers resistance to TYLCV in a heterozygous state, the homozygous state has been reported as offering moderately better resistance.

Breeding

The below protocol detects the *Ty-2* gene locus using a marker. For breeding purposes the gene can be detected in its heterozygous or homozygous. 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 homozygous for *Ty-2*, there

should be no need to confirm the status of their progeny. Similarly, if one of those parents is homozygous and the other negative for *Ty-2,* then there is no need to confirm the status of their progeny. Be sure to confirm the *Ty-2* status when stacking the resistance genes.

Finally, it is not possible to confirm *Ty-2* resistance in a screenhouse nor in the field in Thailand as the virus is not present. This is the real advantage of using markers.

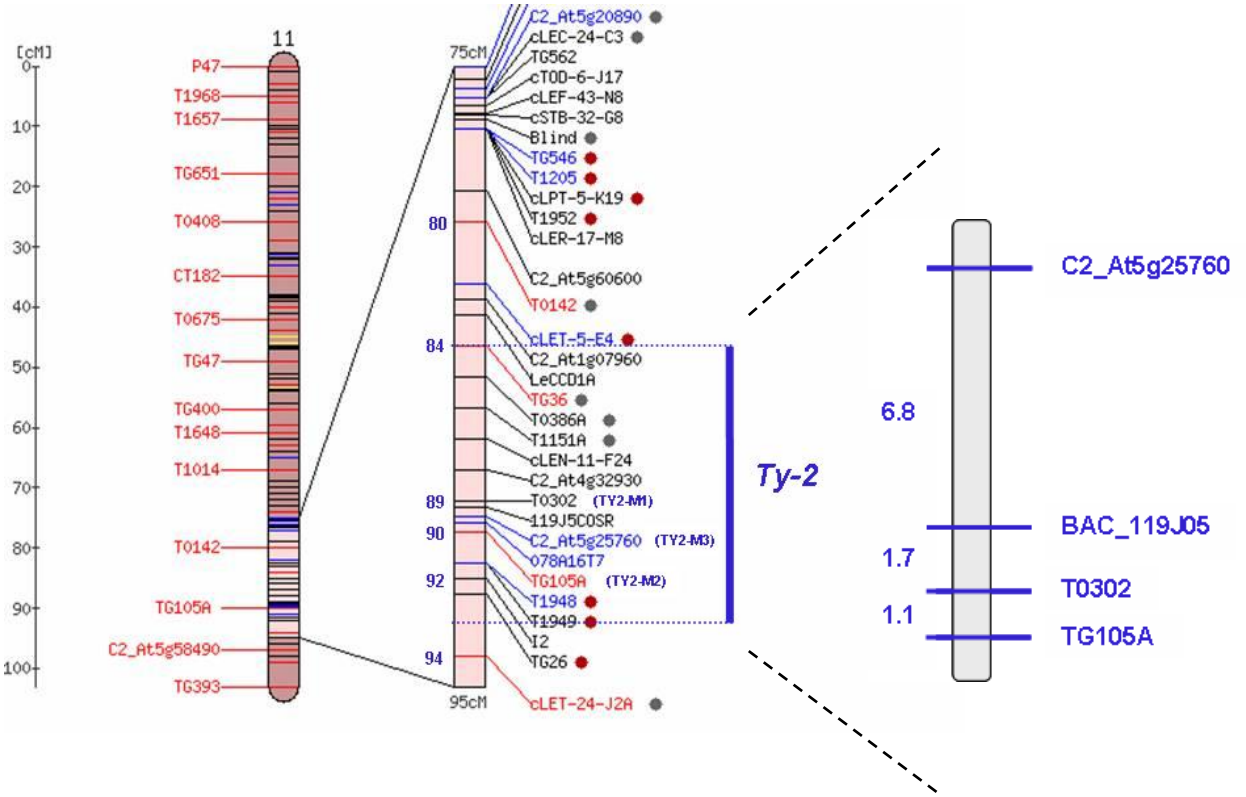

Figure 1. Linkage genetic map of the *Ty-2* region on chromosome 11. Primers for Ty-2(1) were derived from C2_At5g25760 and Ty-2(2) from BAC_119J05.

Protocol Ty-2(2) (Preferred protocol)

1. Tomato leaf DNA extraction

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

1.0 Label each plant.

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

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

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

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

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

1.6 Precipitate the leaf extracts by centrifuging in a microtitre 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.

1.7 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).

1.8 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.

1.9 Store at –20°C or 4°C overnight.

NB. In all cases, a positive control (**known** positive control for *Ty-2*) and negative control (**known** negative control for the *Ty-2*) **MUST** be included. In both cases these should be extracted at the same time as the samples. Controls can be leaf discs stored at -20°C.

Controls: Negative – MH-1 (AVRDC)

Homozygous - H24 (AVRDC)

Heterozygous – breeder inbred line

2. PCR

Primers*

Primer BAC F: 5' AAC TTA CGG CAC CTC AAT TTT TC 3'

Primer BAC R: 5' GTG CCC CCT ATG CAA GTA ATT C 3'

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 BAC F (10 pmole/μl)	0.5	48.5
Primer BAF R (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

** 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
	65°C for 30 seconds
	72°C for 30 seconds
1 cycle:	72°C for 10 minutes

- When the PCR is complete add 2 μl of 6X gel loading buffer to each tube

  6X gel Loading buffer:
  12.5 mg bromophenol blue
  12.5 mg xylene cyanol
  1.875 ml 80% glycerol
  1.5 ml EDTA
  1.665 ml dH_2O

- Run gel. Alternatively, reactions can be stored at 4°C.

3. Restriction enzyme digestion of PCR amplified products

None required.

4. Agarose Gel Electrophoresis

Electrophoresis DNA in 1.5% agarose gels at 100 volt for approximately 40 minutes (this will vary depending on the electrophoresis unit used).

- Prepare 1.5% agarose gel in 0.5X TBE buffer *(TBE 10X: to 800 ml dH_2O add 108g Tris, 55g boric acid, 9.3 g EDTA and make up to 1 litre)* and dissolve by heating in a microwave.
- Cool the molten agarose to 50 – 60°C, pour into the mould, 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 14 μl into the wells. Include the appropriate molecular weight marker as well as the negative and positive controls.
- Run the gel at 100 volt for approximately 40 minutes until the bromophenol blue dye front is within 1 cm of the end of the gel.

Visualisation of DNA using SyberGold or Ethidium Bromide

- Submerge the gel and stain in SyberGold (5 μl stock in 50 ml 0.5X TBE buffer) or ethidium bromide solution (10 mg/ml) for 20 minutes.
- Rinse the gel in dH_2O.
- Visualize the restrictioned amplified DNA fragments under ultraviolet-illumination
- Verify results against DNA molecular weight and positive, negative control.

5. 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).

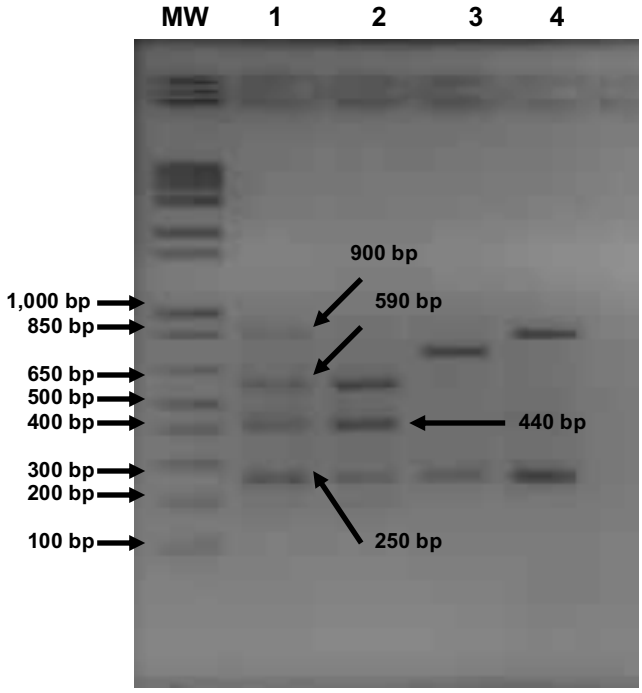

Figure2. Agarose gel electrophoresis of PCR amplified DNA associated *Ty-2* gene detection in tomato. Controls include, Heterozygous resistance (lane 1), Homozygous resistance (lane 2) and susceptible (lane 3 and 4). Three DNA different DNA patterns are possible:

a. **Negative result**: Two bands of 900 and 250 bp indicate an absence of the *Ty-2* resistance gene. Lanes 3 and 4.

b. **Homozygous**: Three bands of 590, 440 and 250 bp indicate the homozygous state. Lane 2.

c. **Heterozygous**: Four bands of 900, 590, 440 and 250 bp indicate the heterozygous state. Lane 1.

6. Summary

- Tomato lines that have **DO NOT contain the *Ty-2 gene*** are "**TYLCV susceptible**".

- Tomato lines that **contain the *Ty-2 gene*** either in its homozygous (three bands at 590, 440 and 250 bp) or heterozygous (four bands at 900, 590, 440 and 250 bp) states are "***Ty-2* resistant**".

- *Ty-2* resistance is only found in a very, very limited number of geographic areas including Taiwan, Israel and some parts of northern Vietnam (but not all) and some parts of southern India (but not all). Ty-2 resistance is not effective in the major tomato growing areas of Thailand, Vietnam, Indonesia, The Philippines and India

Protocol Ty-2(1)

Only use this protocol to repeat negative or non-positive results from the Ty-2(2) 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 microtitre 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.

NB. In all cases, a positive control (**known** positive control for *Ty-2*) and negative control (**known** negative control for the *Ty-2*) **MUST** be included. In both cases these should be extracted at the same time as the samples. Controls can be leaf discs stored at -20°C.

Controls: Negative – MH-1 (AVRDC)

Homozygous - H24 (AVRDC)

Heterozygous – Breeder inbred line

2. PCR

Primers*

Primer Tmy2F: 5' TGG CTC ATC CTG AAG CTG ATA GCG C 3'

Primer Tmy2R: 5' AGT GTA CAT CCT TGC CAT TGA CT 3'

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 Tmy2F (10 pmole/μl)	0.5	48.5
Primer Tmy2R (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	-

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- Add 1 μl of DNA template.

NB: Don't forget the PCR Negative control

** 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

- When the PCR is complete add 2 μl of 6X gel loading buffer to each tube

  6X gel Loading buffer:
  12.5 mg bromophenol blue
  12.5 mg xylene cyanol
  1.875 ml 80% glycerol
  1.5 ml EDTA
  1.665 ml dH_2O

- Run gel. Alternatively reactions can be stored at 4°C.

3. Restriction enzyme digestion of PCR amplified products

None required.

4. Agarose Gel Electrophoresis

Electrophoresis DNA in 1.5% agarose gels at 100 volt for approximately 40 minutes (this will vary depending on the electrophoresis unit used).

- Prepare 1.5% agarose gel in 0.5X TBE buffer *(TBE 10X: to 800 ml dH_2O add 108g Tris, 55g boric acid, 9.3 g EDTA and make up to 1 litre)* and dissolve by heating in a microwave.
- Cool the molten agarose to 50 – 60°C, pour into the mould, 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 14 μl into the wells. Include the appropriate molecular weight marker as well as the negative and positive controls.
- Run the gel at 100 volt for approximately 40 minutes until the bromophenol blue dye front is within 1 cm of the end of the gel.

Visualisation of DNA using SyberGold or Ethidium Bromide

- Submerge the gel and stain in SyberGold (5 μl stock in 50 ml 0.5X TBE buffer) or ethidium bromide solution (10 mg/ml) for 20 minutes.
- Rinse the gel in dH_2O.
- Visualize the restrictioned amplified DNA fragments under ultraviolet-illumination
- Verify results against DNA molecular weight and positive, negative control.

7. Analysis of DNA fragments pattern

a. 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).

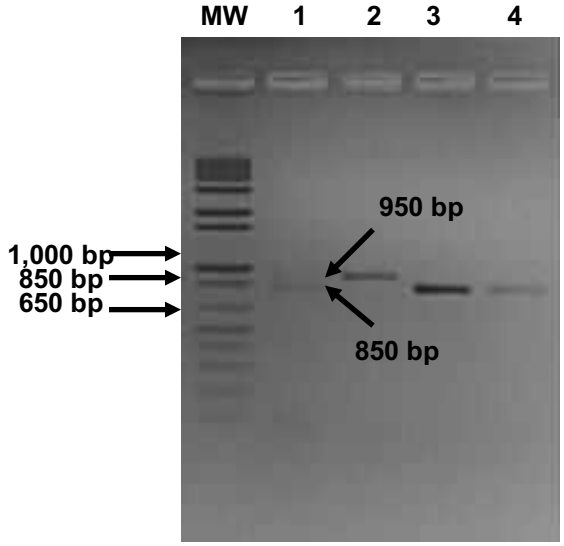

Figure1. Agarose gel electrophoresis of PCR amplified DNA associated *Ty-2* gene detection in tomato. Controls include, Heterozygous resistance (lane 1), Homozygous resistance (lane 2) and susceptible (lane 3 and 4). Three DNA different DNA patterns are possible:

b. **Negative result**: A single band of 850 bp indicates an absence of the *Ty-2* resistance gene. Lanes 3 and 4.

c. **Homozygous**: One band of 950 bp indicates the homozygous state. Lane 2.

d. **Heterozygous**: Two bands of 950 bp (for resistance) and 850 bp (for susceptible) indicates the heterozygous state. Lane 1.

8. Summary

- Tomato lines that have **DO NOT contain the *Ty-2 gene*** are "**TYLCV susceptible**".

- Tomato lines that **contain the *Ty-2 gene*** either in its homozygous (one band at 950 bp) or heterozygous (two bands at 950 bp and 850 bp) states are "***Ty-2* resistant**".

- *Ty-2* resistance is only found in a very, very limited number of geographic areas including Taiwan, Israel and some parts of northern Vietnam (but not all) and some parts of southern India (but not all). Ty-2 resistance is not effective in the major tomato growing areas of Thailand, Vietnam, Indonesia, The Philippines and India

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Trouble Shooting

1. If it is believed that both parents are homozygous for the Ty-2 gene but the progeny is either heterozygous or are segregating for homozygous and heterozygous, then the original assumption of both parents being homozygous is incorrect.

2. On occasions you will observe a faint band or smear associated with the upper 950 bp band. Have you run the samples using a 1.5 or 2% gel?

   Using a 1% gel the 950 and 850 bp bands appear very close to each other and on occasions may not be adequately separated to effectively score the result.

   Firstly, check the positive control and other samples. If the band is of a lower MW than the other heterozygous and homozygous resistant samples, the sample in question is not resistant. If in real doubt, repeat the PCR but also make sure you run the sample using a 1.5 or 2% gel and that the BPB dye is just about to run off the gel.

Additional notes on the development of this protocol

This protocol was developed by AVRDC as part of a collaborative program between APSA members and AVRDC. The result was the primers as listed above. At the end of the project additional primers were developed that were reported by AVRDC as being more efficient.

In our hands, the original set of primers C2_At5g25760 and C2_At5g25760 R3 derived from the BAC clone C2_At5g25760 (protocol Ty-2(1)), resulted in a single band for resistance (950 bp) and a single band for susceptible 850 bp. Heterozygous lines had both bands. On an agarose gel often it is difficult to distinguish the 850 bp band from the 950 bp band and as such **the more recent protocol (Ty-2(2)) using primers BAC 119J05 F1 and BAC 119J05 R1 from BAC clone BAC 119J05 is preferred.** However, in using protocol Ty-2(2) we have found that for some lines, in particular line 01621, results in a band that is neither positive nor negative. As a result it is recommended that all negative results, or results that are not positive, are confirmed using protocol Ty2(1).

It is very important that the protocol be followed exactly as slight variations result in errors.

There are a number of documents (listed below) that are relevant to this protocol. There are other documents associated with this project including agreements, project outlines and progress reports. All are available in the shared Plant Pathology/Protocols folder on the CT exchange server (or see Tom).

Documents:

APSA-AVRDC TY2 Phase 3 Report.doc (see below)
November 2003-August 2004 Progress Report.doc
TY-2 Marker Assay.doc

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31 May 2007

Dear Dr. Sampan,

It was a pleasure meeting you at the APSA-AVRDC workshop. As promised, please find the enclosed Phase III report of the APSA-funded project entitled "Development of a PCR-based screening protocol for detection of a gene conditioning geminivirus tolerance in tomato". Please forward this report to the other APSA contributing members and I would appreciate any feedback and/or comment regarding the project.

Many thanks for APSA's support and I look forward to a continued productive collaboration between APSA and AVRDC.

With best regards,

Robert de la Pena, Ph.D.
Molecular Plant Breeder

Development of a PCR-based screening protocol for detection of a gene conditioning geminivirus tolerance in tomato

Phase III Report

I. Phase I and II Summary

Sequencing of potential probes, and design and synthesis of PCR primers located on the long arm of chromosome 11 were initiated to identify markers closely linked to *Ty-2*. PCR primers for 6 out of 12 markers amplified well but did not show polymorphism in the restriction enzyme panel screen. Two markers (cLET24J2 and TG393) were polymorphic in H24 but not the tolerant AVRDC breeding line that was developed from H24, indicating that the terminal region of the introgression has been lost and the length of *L. hirsutum* DNA has been shortened. Two PCR primers (TG105A and T0302) showed robust amplification and polymorphism either through direct detection of PCR products or digestion by restriction enzyme. The markers and *Ty-2* segregation ratios were not significantly different from the expected F2 segregation. Linkage analysis shows that TG105A and T0302 are tightly linked to each other in the introgressed region of chromosome 11 and *Ty-2* is approximately 10 cM from these markers. TG105A and T0302 were converted into PCR based markers and a *Ty-2* assay protocol based on T0302 that was sent to the APSA members. Two AVRDC F2 populations (CLN2644 x BL1306, CLN2651 x CLN808-5-16-1-12-12-5) and seven selected *Ty-2* homozygous breeding lines were used to confirm the utility of T0302 for MAS of *Ty-2*. Based on TYLCV incidence, 20 out of 22 homozygous lines for T0302 were found to be tolerant to the virus (91%) for the CLN2644 x BL1306 population. The reaction of heterozygous lines was variable indicating that *Ty-2* might be partially dominant. Twenty four out of 26 T0302 homozygous breeding lines were tolerant, confirming close linkage to T0302. Overall, the results indicated that the use of T0302 for MAS provides at least 90% probability of selecting tolerant lines. AVRDC has screened materials from participating APSA members to confirm applicability of T0302 for MAS. An AVRDC-APSA *Ty-2* training workshop was conducted in 13-15 June, 2005 at AVRDC, Taiwan and representatives from several of the APSA companies supporting the project attended. The goals of this training workshop included the use of T0302 for MAS of *Ty-2* and evaluation of germplasm for TYLCV resistance. The workshop also covered presentations and laboratory hands-on activities on AVRDC tomato marker development program.

II. Phase III Result

Detection of *Ty-2* introgression from *L. hirsutum*. Markers from chromosome 11 were screened to identify the introgressed *Ty-2* region from *L. hirsutum* (Table 1). Most markers were not polymorphic based on direct PCR amplification. Restriction enzymes (if available) were then used to identify polymorphism. At this stage if the marker is monomorphic, the bands were cloned from the resistant and susceptible parents for sequencing. Some clones have very high sequence diversity between the two parents while 0, 1, or 2 SNPs were detected in some markers. One terminal end of the introgression was identified using cLET24J2 marker, however the other end can not be ascertained based on the markers screened.

Table 1. Summary of marker screening for *Ty-2*

Marker	Chromosome Position (cM)	PCR Amplification	Enzyme	Gel Polymorphism (bp)	Sequence Polymorphism
cLEC24C3	76.0	+	EcoRI	-	
Blind	76.7	+	-	-	
T1952	77.0	+	-	-	
T1205	77.0	+	MnlI	Multiple	2 SNPs
C2_At5g60600	79.0	+	Rsa I	-	
TG30	82.0	+	-	-	
C2_At1g07960	82.5	+	-	-	
TG36	84.0	-	-	-	
T0386	85.0	+	-	-	
T1151	86.0	+	HinfI	410/310, not chromosome 11	13 SNPs (CAPS)
T0302	89.0	+	-	850/950	INDEL
BAC_119J05	89.2	+	-	440/590/900	SNP, INDEL
C2_At5g25760	89.5	+	DraI	650/620	40 SNPs, INDEL
TG105A	90.0	+	TaqI	330/220	SNP (CAPS)
T1948	91.0	-	-	-	
T1949	91.0	+	-	-	
I2	91.5	+	CtoI/DraI	-	
TG26	92.0	+	-	-	
cLET24J2	94.0	+	-	400/250, not introgressed region	
TG393	103.0	+	DpnII	-	

Mapping of the *Ty-2* region on chromosome 11. Five polymorphic markers were segregating in the CLN2123A x BL1306 population. Based on linkage analysis, T0302, BAC_119J05, C2_At5g25760, and TG105A were closely linked to each other while T1151 was unlinked (Figure 1). The total map distance of the 4 markers is 9.2 cM. Linkage of these markers to *Ty-2* has to be confirmed. *Ty-2* is losing its resistance at high disease pressures at AVRDC field sites. This makes it difficult to accurately map *Ty-2* on chromosome 11.

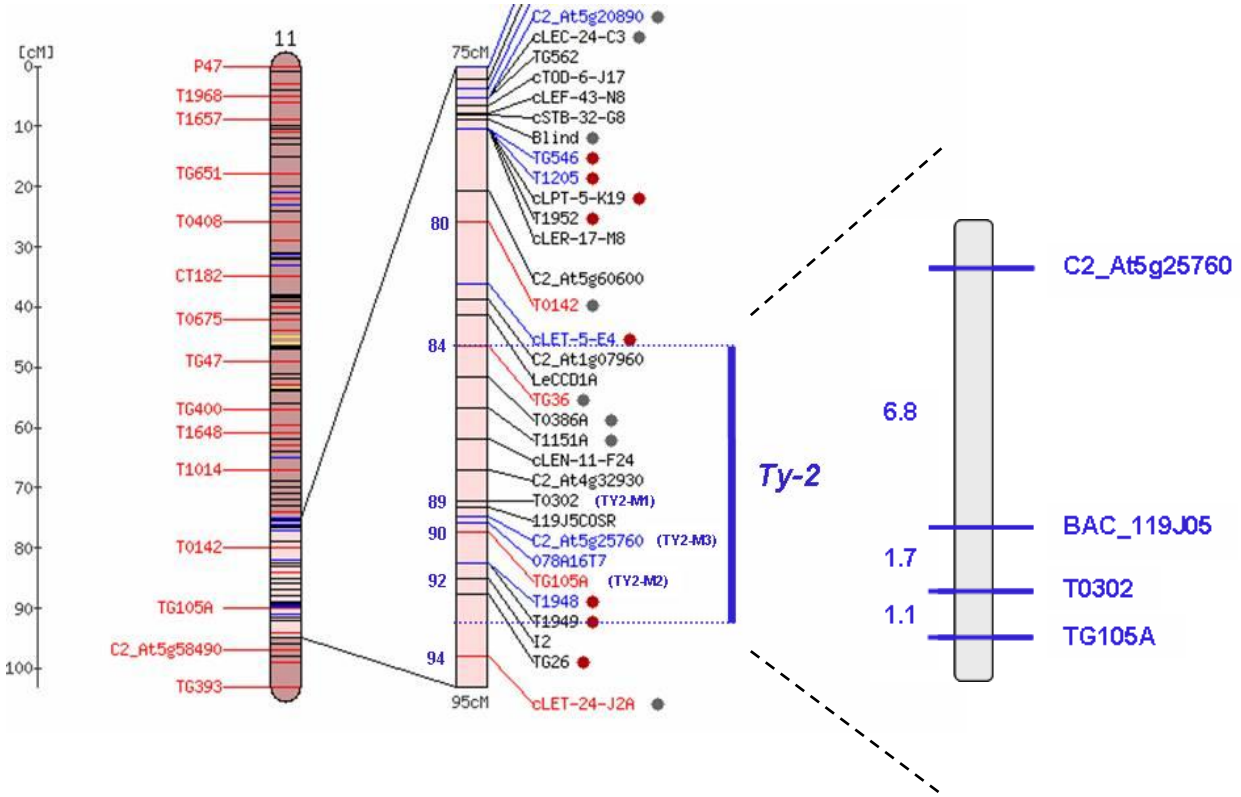


Figure 1. Linkage genetic map of the *Ty-2* region on chromosome 11

Development of PCR protocol for MAS using BAC_119J05 and C2_At5g25760. PCR primers were designed from sequences of clones BAC_119J05 and C2_At5g25760. Forty SNPs and two INDELS were identified in C2_At5g25760. Primers were designed to amplify the largest inserted sequence of clone C2_At5g25760 to enhance gel separation of fragments. Association of homozygous *Ty-2* lines to BAC_119J05 and C2_At5g25760 markers were determined and shown in Table 2. Both markers showed around 93-94% accuracy in detecting resistant genotypes. The probability of detecting resistant types could be increased by using two or more markers. The utility of these markers has to be further tested in a large number of genotypes to validate its utility and accuracy estimates. The procedures for detecting *Ty-2* using BAC_119J05 and C2_At5g25760 markers are described in Protocols 1 and 2.

Table2. Probability of detecting resistant *Ty-2* alleles

Marker	Resistant	Susceptible	% Accuracy
BAC_119J05	29	2	94%
C2_At5g25760	41	3	93%
T0302	32	4	89%
TG105A	33	2	94%

Protocol 1. Marker-assisted selection of *Ty-2* using BAC 119J05

Primer sequence

BAC_119J05 F1: AAC TTA CGG CAC CTC AAT TTT TC
BAC_119J05 R1: GTG CCC CCT ATG CAA GTA ATT C

PCR cocktail

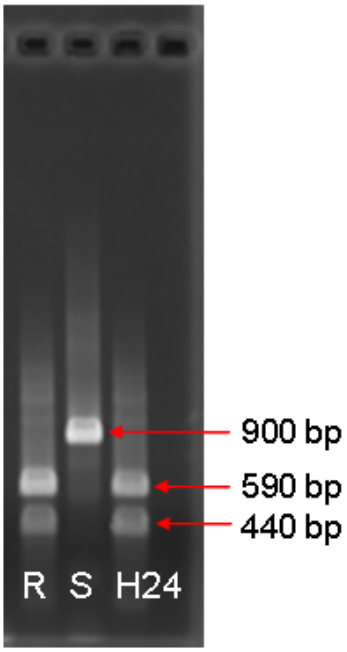
Primer (10μM) F	1.25 μl
Primer (10μM) R	1.25 μl
10X PCR buffer	2.50 μl (with 15mM $MgCl_2$)
dNTPs (2.5mM)	2.25 μl
Taq polymerase	0.50 μl (0.5unit/reaction)
ddH_2O	12.25 μl
DNA (50-100ng)	5.00 μl
	25.00 μl total

PCR program

1 cycle:	94°C	5 min
35 cycles:	94°C	30 sec
	60°C	1 min
	72°C	2 min
1 cycle:	72°C	5 min
Storage:	10°C	

Gel visualization

Use 1.5-2.0 % agarose gel
R (*L. hirsutum*, CLN2123A) amplicon: 440, 590 bp
S (*L. esculentum*, BL1306) amplicon: 900 bp



Protocol 2. Marker-assisted selection of *Ty-2* using C2_At5g25760

Primer sequence

C2_At5g25760 F1 : AAGAGAGCGAAGATACAGATTTGG
C2_At5g25760 R3 : GGGGCAATGTCAAACCTCT

PCR cocktail

Primer (10μM) F	1.25 μl
Primer (10μM) R	1.25 μl
10X PCR buffer	2.50 μl (with 15mM $MgCl_2$)
dNTPs (2.5mM)	2.25 μl
Taq polymerase	0.50 μl (0.5unit/reaction)
ddH_2O	12.25 μl
DNA (50-100ng)	5.00 μl
	25 μl total

PCR program

1 cycle:	94°C	5 min
35 cycles:	94°C	30 sec
	55°C	1 min
	72°C	2 min
1 cycle:	72°C	5 min
Storage:	10°C	

Gel visualization

Use 1.5% agarose gel
R (*L. hirsutum*, CLN2123A) amplicon: 620 bp
S (*L. esculentum*, BL1306) amplicon: 650 bp