

Technical Committee
TC/53/27
**Fifty-Third Session
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PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO
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1. The TC, at its fifty-second session held in Geneva from March 14 to 16, 2016, had agreed to include discussions on the partial revision of the Test Guidelines for Tomato (document TG/44/11 Rev.), characteristic 57 “Resistance to Tomato yellow leaf curl virus (TYLCV)”, at the fiftieth session of the Technical Working Party for Vegetables (TWV) to be held in Brno, Czech Republic, from June 27 to July 1, 2016, on the basis of a document to be prepared by an expert from the European Union (see document TC/52/29 Rev. “Revised Report”, paragraph 197).

2. At its fiftieth session the TWV considered a proposal for a partial revision of the Test Guidelines for Tomato on the basis of documents TG/44/11 Rev. and TWV/50/20 “Partial Revision of the Test Guidelines for Tomato (Document TG/44/11 Rev.)” and proposed to revise the Test Guidelines for Tomato as follows (see document TWV/50/25 “Report”, paragraph 90):

- (a) Revision of Characteristic 57 “Resistance to Tomato yellow leaf curl virus (TYLCV)”:
 - (i) Revision of the example varieties for state 1 “absent”
 - (ii) Revision of the current methodology for TYLCV as outlined in Ad. 57 (i) , and to add an alternative methodology using white fly inoculation as outlined in Ad. 57 (ii)

3. The proposed changes are presented below in highlight and underline (insertion) and ~~striethrough~~ (deletion).

Proposal for a Revision of the Example Variety for state 1 “absent”

Current Wording:

| | English | français | deutsch | español | Example Varieties Exemples Beispielssorten Variedades ejemplo | Note/ Nota |
|---------------|--|---|--|---|--|---------------|
| 57. VG | Resistance to Tomato yellow leaf curl virus (TYLCV) | Résistance au virus des feuilles jaunes en cuillère de la tomate (TYLCV) | Resistenz gegen gelbes Tomatenblatt-rollvirus (TYLCV) | Resistencia al virus del rizado amarillo de la hoja del tomate (TYLCV) | | |
| QL | absent | absente | fehlend | ausente | Montfavet H 63.5 | 1 |
| | present | présente | vorhanden | presente | Anastasia, Mohawk, TY 20 | 9 |

Proposed new wording:

| | English | français | deutsch | español | Example Varieties Exemples Beispielssorten Variedades ejemplo | Note/ Nota |
|---------------|--|---|--|---|--|---------------|
| 57. VG | Resistance to Tomato yellow leaf curl virus (TYLCV) | Résistance au virus des feuilles jaunes en cuillère de la tomate (TYLCV) | Resistenz gegen gelbes Tomatenblatt-rollvirus (TYLCV) | Resistencia al virus del rizado amarillo de la hoja del tomate (TYLCV) | | |
| QL | absent | absente | fehlend | ausente | Montfavet H 63.5 <u>Marmande, Moneymaker</u> | 1 |
| | present | présente | vorhanden | presente | Anastasia, Mohawk, TY 20 | 9 |

Proposal for a Revision of the current methodology for TYLCV as outlined in Ad. 57 (i) , and to add an alternative methodology using white fly inoculation as outlined in Ad. 57 (ii)

Current wording:

Ad. 57: Resistance to Tomato yellow leaf curl virus (TYLCV)

1. Pathogen Tomato yellow leaf curl virus
2. Quarantine status yes
3. Host species *Solanum lycopersicum*
4. Source of inoculum -
5. Isolate -
8. Multiplication inoculum
- 8.6 Harvest of inoculum symptomatic leaves may be stored at -70°C
9. Format of the test
- 9.1 Number of plants per genotype 20 plants
- 9.2 Number of replicates 1 replicate
- 9.3 Control varieties
- Susceptible Montfavet H 63.5
- Resistant TY 20, Anastasia, Mohawk
- 9.5 Test facility field with natural disease pressure
- 9.9 Special measures prevent spread of white-flies
10. Inoculation
- 10.3 Plant stage at inoculation 6-12 weeks (adult plants)
- 10.4 Inoculation method vector (Bemisia white-flies carrying TYLCV)
- 10.7 Final observations 1-2 months after inoculation
11. Observations
- 11.1 Method visual
- 11.2 Observation scale symptoms: leaf yellowing and curling
- 11.3 Validation of test evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties
- absent [1] severe symptoms
- present [9] no or mild symptoms
13. Critical control points:
TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate. TYLCV is on the EPPO alert list. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).

Proposed new wording:

Ad. 57: Resistance to Tomato yellow leaf curl virus (TYLCV)

(i) agroinoculation method

1. Pathogen Tomato yellow leaf curl virus (TYLCV) IL strain. (See note below)
2. Quarantine status yes (see 13.)
3. Host species *Solanum lycopersicum*
4. Source of inoculums Dr. Eduardo R. Bejarano, Plant Genetics Laboratory, IHSM UMA-CSIC)¹
5. Isolate Alm:Pep:99, strain IL
6. Establishment isolate identity
7. Establishment pathogenicity
8. Multiplication inoculum
- 8.1 Multiplication medium YEP/Kanamycin.
- 8.2 Multiplication variety
- 8.3 Plant stage at inoculation 3-4 leaf
- 8.4 Inoculation medium YEP

¹ Source of inoculum; HMS UMA (CSIC) edu_rodri@uma.es; INIA Cardaba@inia.es

- 8.5 Inoculation method Stem puncture agroinfiltration. Plant agroinoculation is carried out using *Agrobacterium tumefaciens* transformed with plasmids containing the infectious clones (Morilla, et al. 2005. *Phytopathology* 95: 1089-1097)
- 8.6 Harvest of inoculums
- 8.7 Check of harvested inoculums
- 8.8 Shelflife/viability inoculums..... *A. tumefaciens* stocks are maintained frozen at -80°C in 15-20% glycerol for long term storage. Cultures to be stored are typically started from a single colony and grown in 5 ml YEP +2.5 µl kanamycin (100mg/ml) during 48 h at 28°C.
9. Format of the test
- 9.1 Number of plants per genotype 20
- 9.2 Number of replicates 2
- 9.3 Control varieties..... Susceptible: Moneymaker, Marmande,
..... Resistant: Delyca, Montenegro, Anastasia, TY20, Mohawk
- 9.4 Test design
- 9.5 Test facility..... Glasshouse or climatic chamber with permission to confined use of OGM, confinement level 1 (N-1).
- 9.6 Temperature 23-25°C
- 9.7 Light 16 h
- 9.8 Season.....
- 9.9 Special measures Permission to confined use of OGM, at least level 1 (N-1)
10. Inoculation
- 10.1 Preparation inoculums..... Streak the surface of the frozen *A. tumefaciens* stock tube and submerge in 5 ml YEP+2.5 µl kanamycin (100mg/ml) during 48 h at 28°C. Shaking is needed. Take 100 µl and place them into 100 ml YEP and 50 µl kanamycin (100mg/ml). Shake 48 h at 28°C. Centrifuge the saturated culture for 20 min at 3500 rpm and discard supernatant.
- 10.2 Quantification inoculums Dissolve in sterile deionize water to a final OD₆₀₀ of 1.
- 10.3 Plant stage at inoculation 3-4th leaf
- 10.4 Inoculation method Take up into a 1 ml syringe with a 27-gauge needle and few drops (about 20 µl of the culture) were deposited on 10-15 puncture wounds made with the needle into the stem of test tomato plants. Maintain on ice while inoculating plants.
- 10.5 First observation 20 days post inoculation
- 10.6 Second observation 30 dpi
- *10.7 End of test – Final observation..... 45 dpi
11. Observations
- 11.1 Method..... Visual
- 11.2 Observation scale Symptoms: leaf yellowing and curling
- 11.3 Validation of test evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12. Interpretation of data in terms of UPOV characteristic states
- absent [1] severe symptoms
- present [9] no symptoms
13. Critical control points:
- TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate.
- TYLCV-IL is the strain most widely spread worldwide. With this strain, symptoms do not appear in varieties with Ty-1 and Ty-2.
- TYLCV is on the EPPO alert list. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).

Ad. 57: Resistance to Tomato yellow leaf curl virus (TYLCV)

(ii) White fly inoculation method

1. Pathogen Tomato yellow leaf curl virus (TYLCV) IL strain
2. Quarantine status yes (see 13.)
3. Host species *Solanum lycopersicum*

4. Source of inoculum-Spain
5. Isolate-TYLCV-IL La Mayora
8. Multiplication inoculumWhite flies
8.6 Harvest of inoculum
9. Format of the test
9.1 Number of plants per genotype20
9.2 Number of replicates..... Two replicates
9.3 Control varieties
Susceptible:Moneymaker, Marmande,

Resistant:Delyca, Montenegro, Anastasia, TY20, Mohawk
9.5 Test facilityGreenhouse/plastic tunnel
9.9 Special measuresprevent spread of white-flies
10. Inoculation
10.3 Plant stage at inoculation2-4 weeks
10.4 Inoculation methodvector (Bemisia white-flies carrying TYLCV-IL)
10.7 Final observations1-2 months after inoculation
11. Observations
11.1 Method.....visual
11.2 Observation scaleSymptoms: leaf yellowing and curling
11.3 Validation of testevaluation of variety resistance should be calibrated with results of
resistant and susceptible controls
12. Interpretation of data in terms of UPOV characteristic states
absent [1] severe symptoms
present [9] no or mild symptoms
13. Critical control points:
TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with
a temperate climate.
TYLCV-IL is the strain most widely spread worldwide. With this strain, symptoms do not appear in varieties
with Ty-1 and Ty-2.
Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl
Sardinia virus (TYLCSV).
Source of inoculum: IHSM, CSIC guillamon@eelm.csic.es or INIA cardaba@inia.es

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