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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL WORKING PARTY FOR VEGETABLES

Fiftieth Session
Brno, Czech Republic, June 27 to July 1, 2016

PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO
(DOCUMENT TG/44/11 REV.)

Document prepared by an expert from the European Union

Disclaimer: this document does not represent UPOV policies or guidance

1. The TC 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 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. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Tomato (document TG/44/11 Rev.).

3. The following changes are proposed:

(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)

4. The proposed changes are presented below in highlight and underline (insertion) and ~~strikethrough~~ (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~~ Marmande, Moneymaker	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 (i): Resistance to Tomato yellow leaf curl virus (TYLCV)

1. Pathogen ..... Tomato yellow leaf curl virus (TYLCV) IL strain. (See note below)
2. Quarantine status ..... yes
3. Host species ..... *Solanum lycopersicum*
4. Source of inoculums ..... Dr. Eduardo R. Bejarano, Plant Genetics Laboratory, HMS 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 -80ºC in 15-20% glicerol 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 28ªC.
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, confinment 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 28ªC. Shaking is needed. Take 100 µl and place them into 100 ml YEP and 50 µl kanamycin (100mg/ml). Shake 48 h at 28ºC. 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 OD_{600} 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 worldwhile. 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 (ii): Resistance to Tomato yellow leaf curl virus (TYLCV) White fly inoculation

1. Pathogen .....Tomato yellow leaf curl virus (TYLCV) IL strain
2. Quarantine status .....yes
3. Host species .....*Solanum lycopersicum*
4. Source of inoculum.....-Spain
5. Isolate .....-TYLCV-IL La Mayora

8. Multiplication inoculum.....White flies
8.6 Harvest of inoculum.....
9. Format of the test
9.1 Number of plants per genotype 20
9.2 Number of replicates……Two replicates
9.3 Control varieties
Susceptible: .....Moneymaker, Marmande,

Resistant: .....Delyca, Montenegro, Anastasia, TY20, Mohawk
9.5 Test facility.....Greenhouse/plastic tunnel
9.9 Special measures .....prevent spread of white-flies
10. Inoculation
10.3 Plant stage at inoculation .....2-4 weeks
10.4 Inoculation method .....vector (Bemisia white-flies carrying TYLCV-IL)
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 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 worldwhile. 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, INIA cardaba@inia.es

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