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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR VEGETABLES

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

Document prepared by an expert from the Netherlands

1. The Technical Working Party for Vegetables, at its forty-seventh session, held in Veliko Tarnovo from July 5 to 9, 2010, agreed to propose the adoption of the Test Guidelines for Tomato on the basis of document TG/44/11(proj.3), subject to an item being included at its forty-fifth session for a possible partial revision in order to consider:

(a) further discrimination within varieties with red colored fruits (see document TG/44/11(proj.3), Chars. 36 and 37;
(b) revised format for disease resistance characteristics; and
(c) gene-specific marker method for examination of resistance to Tomato Spotted Wilt topovirus (TSWV) - Race 0.

(see document TWV/44/34, paragraph 77)

2. The Technical Committee (TC), at its forty-seventh session, held in Geneva from April 4 to 6, 2011, adopted the Test Guidelines for Tomato on the basis of document TG/44/11(proj.5) and the amendments specified in Annex II, paragraph 76 to document TC/47/26 “Report on the Conclusions”, and the linguistic changes recommended by the Enlarged Editorial Committee (TC-EDC), subject to the Council adopting the necessary revision of document TGP/7 as set out in paragraph 99 of document TC/47/26.

3. The TC, at its forty-seventh session considered document TC/47/23 “Revision of Document TGP/12: Disease nomenclature and disease resistance characteristics”.

4. The TC agreed that document TC/47/23, Annex I, should be developed further with regard to states of expression for quantitative disease resistance characteristics.

5. With regard to the proposed standard disease resistance protocols in Section 2.4 of Annex I to document TC/47/23, as reproduced in Annex I to this document, the TC agreed that:

- the information items that were not asterisked in the protocol should not be elaborated in detail in the Test Guidelines and should be replaced by a reference to the contact details for UPOV members that would be able to provide such information on request. The TC agreed that the asterisk symbol should be replaced in order to avoid confusion.

- the explanations for disease resistance characteristics in the Test Guidelines should refer to published methods rather than reproducing the methods in the Test Guidelines.

- it was important to recall that authorities could arrange for tests to be conducted by specialized laboratories and could also use cooperation with other UPOV members in order to address situations where the DUS testing center did not have suitable facilities for conducting the test, or was prevented from conducting such tests because of phytosanitary restrictions. It agreed that it would be useful for document TGP/12 to address such issues and agreed that Mr. Sergio Semon (European Union) should coordinate with Mr. Kees van Ettekoven (Netherlands) the preparation of document TGP/12 for the TWP sessions in 2011.

6. The TC noted the proposals concerning the nomenclature of pathogens, as set out in Annex II to document TC/47/23.

7. The TC agreed that the proposal concerning explanations for disease resistance characteristics in Test Guidelines, as set out in Section 2.4 Annex I to this document.

Partial Revision of Test Guidelines for Tomato

8. It is proposed to revise the Test Guidelines for Tomato (document TG/44/11) in order to consider a revised format for disease resistance characteristics and gene-specific marker method for examination to Tomato topovirus (TSWV)-Race 0.

9. Annex II to this document contains the proposal for a revised format of explanations of disease resistance characteristics in the Test Guidelines for Tomato.

[Annex I follows]

ANNEX I

EXPLANATIONS FOR DISEASE RESISTANCE CHARACTERISTICS IN TEST GUIDELINES:

Proposal developed by experts from the Netherlands

It is proposed that Section I, 2. "Disease Resistance", of document TGP/12/1, be amended by replacing "2.4 Explanations for disease resistance characteristics in Test Guidelines" with the following text:

2.4 Explanations for disease resistance characteristics in Test Guidelines

2.4.1 Where disease resistance characteristics are included in Test Guidelines, the following information should be provided in Chapter 8 "Explanations on the Table of Characteristics" in the form of a standard disease resistance protocol as set out below. This standard resistance protocol is guidance and not a strict prescription. It is not only advised to use the subjects mentioned, it also is advised to use the same order of the subjects. In order to increase the legibility and use of the protocols it is also advised to restrict the number of extra topics.

STANDARD RESISTANCE PROTOCOL
*compulsory

* 1. Pathogen
2. Quarantine status
* 3. Host species
* 4. Source of inoculum
* 5. Isolate
6. Establishment isolate identity
7. Establishment pathogenicity
8. Multiplication inoculum
8.1 Multiplication medium
8.2 Multiplication variety
8.3 Plant stage at inoculation
8.4 Inoculation medium
8.5 Inoculation method
8.6 Harvest of inoculum
8.7 Check of harvested inoculum
8.8 Shelflife/viability inoculum

9. Format of the test
* 9.1 Number of plants per genotype
* 9.2 Number of replicates
* 9.3 Control varieties
9.4 Test design
9.5 Test facility
9.6 Temperature
9.7 Light
9.8 Season
9.9 Special measures

10. Inoculation
10.1 Preparation inoculum
10.2 Quantification inoculum
* 10.3 Plant stage at inoculation
* 10.4 Inoculation method
10.5 First observation
10.6 Second observation
* 10.7 End of test

11. Observations
* 11.1 Method
* 11.2 Observation scale
* 11.3 Validation of test
11.4 Off-types

* 12. Interpretation of data in terms of UPOV characteristic states

13. Critical control points:

[Annex II follows]

ANNEX II

Ad 46: Resistance to *Meloidogyne incognita* (Mi)

1. Pathogen ***Meloidogyne incognita***

3. Host species .....*Lycopersicon esculentum*
4. Source of inoculum .....Naktuinbouw (NL) or GEVES (F)
5. Isolate .....non-resistance breaking
6. Establishment isolate identity ....use rootstock or tomato standards
7. Establishment pathogenicity .....use susceptible rootstock or tomato standard

8. Multiplication inoculum

8.1 Multiplication medium .....living plant
8.2 Multiplication variety.....Delito (resistant to powdery mildew)
8.3 Plant stage at inoculation.....10.3
8.5 Inoculation method.....10.4
8.6 Harvest of inoculum.....root systems are cut with scissors into pieces of about 1 cm length
8.7 Check of harvested inoculum...visual check for presence of root knots
8.8 Shelf life/viability inoculum.....1 day

9. Format of the test

9.1 Number of plants per genotype 20 plants
9.3 Control varieties
Susceptible: .....Clairvil, Casaque Rouge
Moderately resistant : ..... Madyta, "Anahu x Monalbo"
Resistant: .....Anabel, Anahu
9.4 Test design .....include standard varieties
9.5 Test facility .....greenhouse or climate room
9.6 Temperature .....not over 28° C
9.7 Light .....at least 12 h per day

10. Inoculation

10.1 Preparation inoculum .....small pieces of diseased root mixed with soil
.....mix soil and infested root pieces
10.2 Quantification inoculum.....soil: root ratio = 8:1
10.3 Plant stage at inoculation .....seed
10.4 Inoculation method .....plants are sown in infested soil
10.7 End of test .....28 to 45 days after inoculation

11. Observations

11.1 Method .....root inspection
11.2 Observation scale .....Symptoms:
.....Galling, root malformation,
.....growth reduction, plant death
.....1-10 galls per root system may be counted
11.3 Validation of test.....on standards
11.4 Off-types.....resistant varieties may have a few plants with a few galls

12. Interpretation of data in terms of UPOV characteristic states

[1] severe symptoms
[2] mild or no symptoms

13. Critical control points:

Avoid rotting of roots; high temperature causes breakdown of resistance

Literature references

Laterrot, H., 1973: Sélection de variétés de Tomate résistantes aux Meloidogyne, OEPP/EPPO Bulletin 3(1): 89.92.

Ad 47: Resistance to *Verticillium dahliae* (Vd)

1. Pathogen ***Verticillium dahliae*** (see note below)
3. Host species.....*Lycopersicon esculentum*
4. Source of inoculum .....Naktuinbouw (NL) and GEVES (*F*)
5. Isolate .....Race 0

8. Multiplication inoculum
8.1 Multiplication medium.....Potato Dextrose Agar, Medium "S" of Messiaen
8.4 Inoculation medium.....Czapek Dox broth, 20-25°C, in darkness
8.6 Harvest of inoculum.....3-7 d-old aerated culture
.....filter through double muslin cloth
8.7 Check of harvested inoculum...spore count; adjust to 10^6 per ml
8.8 Shelf life/viability inoculum.....1 d at 4°C

9. Format of the test
9.1 Number of plants per genotype 35 seed for 24 plants
9.3 Control varieties
Susceptible ..... Marmande, Flix, Planet
Weakly resistant ..... Monalbo x Marmande verte
Resistant ..... Monalbo, Elias
9.4 Test design.....22 plants inoculated, 2 blanks
9.5 Test facility.....greenhouse or climate room
9.6 Temperature .....25°C for germination, 20-22°C after inoculation
9.7 Light .....16 h or longer

10. Inoculation
10.1 Preparation inoculum .....aerated, liquid culture (8.4)
10.2 Quantification inoculum.....count spores, adjust to 10^6 per ml
10.3 Plant stage at inoculation.....cotyledon to 3rd leaf
10.4 Inoculation method.....roots are immersed for 4 to 15 min in spore suspension.
10.5 First observation.....14 d after inoculation
10.6 Second observation .....21 d after inoculation
10.7 End of test.....21-33 d after inoculation

11. Observations
11.1 Method .....visual
11.2 Observation scale .....growth retardation, wilting, chlorosis, and vessel browning
11.3 Validation of test.....compare

12. Interpretation of data in terms of UPOV characteristic states
[1] severe symptoms
[9] no or mild symptoms

13. Critical control points
All symptoms may be present in resistant varieties, but the severity will be distinctly less than in susceptible varieties. Usually resistant varieties will show significantly less growth retardation then susceptible varieties. Observation of vessel browning is important for diagnosis. Usually, vessel browning will not extend to the 1st leaf in resistant varieties. Many hybrid varieties are heterozygous and appear to have a relatively weak resistance in the biotest.

Note: Resistance to *V. dahliae* based in the Ve gene is also effective to *V. albo-atrum*. Isolates of both fungal species may be used to evaluate the UPOV characteristic "Resistance to *V. dahliae*" as long as the isolate belongs to the non-Ve breaking race 0. Resistance-breaking isolates have been described in both species.

Literature references Denby, L. G., Wooliams, G. E., 1962: The Development of Verticillium Resistant Strains of Established Tomato Varieties, Canadian Journal Plant Science 42,681-685.

Ad 48: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)

1. Pathogen ***Fusarium oxysporum* f. sp. *lycopersici***
3. Host species.....*Lycopersicon esculentum*
4. Source of inoculum .....Naktuinbouw (NL) and GEVES (*F*)
5. Isolate .....Race 0 (ex 1), 1 (ex 2) and 2 (ex 3)
.....Individual strains may vary in pathogenicity
.....Long term storage:-80°C in 20% glycerol
6. Establishment isolate identity.....use differential varieties (see 9.3)
7. Establishment pathogenicity .....on suscpetible tomato varieties
8. Multiplication inoculum
8.1 Multiplication medium.....Potato Dextrose Agar, Medium "S" of Messiaen
8.4 Inoculation medium.....Czapek-Dox culture
8.5 Inoculation method.....immersion of roots in spore suspension, 5-15 min
8.6 Harvest of inoculum.....7 d-old aerated culture
.....filter through double muslin cloth
8.7 Check of harvested inoculum...spore count; adjust to 10^6 per ml
8.8 Shelf-life/viability inoculum ....4-8 h, keep cool to prevent spore germination
9. Format of the test
9.1 Number of plants per genotype at least 20
9.3 Control varieties for the test with <u>race 0 (ex 1)</u>
Susceptible.....Marmande, Marmande verte, Resal
Resistant for race 0 only .....Marporum, Larissa, "Marporum x Marmande verte"
Resistant for race 0 and 1 .....Motelle, Gourmet, Mohawk
Control varieties for the test with <u>race 1 (ex 2)</u>
Susceptible.....Marmande verte, Cherry Belle, Roma
Resistant for race 0 only .....Marporum, Ranco
Resistant for race 0 and 1 .....Tradiro, Odisea
Remark .....Ranco is slightly less resistant than Tradiro
9.4 Test design.....35 seeds for 24 plants, including 2 blanks
9.5 Test facility.....glasshouse or climate room
9.6 Temperature .....24-28°C (severe test, with mild isolate)
.....20-24°C (mild test, with severe isolate)
9.7 Light .....at least 16 hours per day
9.8 Season.....all seasons
9.9 Special measures .....slightly acidic peat soil is optimal;
.....keep soil humid but avoid water stress
10. Inoculation
10.1 Preparation inoculum .....aerated culture 7-10 days
10.2 Quantification inoculum.....spore count, adjust to 10^6 spores per ml
10.3 Plant stage at inoculation.....10-18 d, cotyledon to first leaf
10.4 Inoculation method.....roots and hypocotyls are immersed in spore suspension
.....for 5-15 min; trimming of roots is an option
10.5 First observation.....14 days after inoculation
10.7 End of test.....21 days after inoculation
11. Observations
11.1 Method .....visual
11.2 Observation scale .....Symptoms:
.....growth retardation, wilting, yellowing,
.....vessel browning extending above cotyledon
11.3 Validation of test.....on standards

12. Interpretation of data in terms of UPOV characteristic states

[1] severe symptoms
[9] mild or no symptoms

13. Critical control points

Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature. Standards near borderline R/S are essential to compare between labs.

Literature references

Laterrot, H., 1972: Sélection de tomates résistantes à Fusarium oxysporum f. sp. lycopersici, Phytopathologia Mediterranea, Volume XI, No. 3, p. 154-158.

Ad 49: Resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (For)

1. Pathogen ***Fusarium oxysporum* f. sp. *radicis-lycopersici***

3. Host species ..... *Lycopersicon esculentum*
4. Source of inoculum ..... Naktuinbouw (NL) and GEVES (*F*)
5. Isolate ..... -
7. Establishment pathogenicity ..... symptoms on susceptible tomato

Multiplication inoculum

8.1 Multiplication medium ..... Potato Dextrose Agar, Medium "S" of Messiaen
8.4 Inoculation medium ..... Czapek-Dox culture
8.5 Inoculation method ..... immersion of roots in spore suspension, 5-15 min
8.6 Harvest of inoculum ..... 7 d-old aerated culture
..... filter through double muslin cloth
8.7 Check of harvested inoculum ... spore count; adjust to 10^6 per ml
8.8 Shelf life/viability inoculum ..... 4-8 h, keep cool to prevent spore germination

9. Format of the test

9.1 Number of plants per genotype at least 20
9.3 Control varieties
Susceptible: ..... Motelle, Moneymaker
Resistant: ..... Momor, "Momor x Motelle"
Remark: ..... "Momor x Motelle" has slightly weaker resistance than Momor
9.4 Test design ..... 35 seeds for 24 plants, including 2 blanks
9.5 Test facility ..... glasshouse or climate room
9.6 Temperature ..... 24-28°C (severe test, with mild isolate)
..... 20-24°C (mild test, with severe isolate)
9.7 Light ..... at least 16 hours per day
9.8 Season ..... all seasons
9.9 Special measures ..... slightly acidic peat soil is optimal;
..... keep soil humid but avoid water stress

10. Inoculation

10.1 Preparation inoculum ..... aerated culture 7-10 days
10.2 Quantification inoculum ..... spore count, adjust to 10^6 spores per ml
10.3 Plant stage at inoculation ..... 12-18 d, cotyledon to third leaf
10.4 Inoculation method ..... roots and hypocotyls are immersed in spore suspension
..... for 5
10.5 First observation ..... 14 days after inoculation
10.7 End of test ..... 14-21 days after inoculation

11. Observations

11.1 Method ..... visual; a few plants are lifted at the end of the test
11.2 Observation scale ..... Symptoms:
..... Plant death
..... Growth retardation caused by root degradation
..... Root degradation

12. Interpretation of data in terms of UPOV characteristic states

[1] symptoms
[9] no symptoms

13. Critical control points

Temperature should never exceed 27°C during the test period; frequent renewal of races may be needed because of loss of pathogenicity

Ad 50: Resistance to *Fulvia fulva* (Ff)

1. Pathogen ***Fulvia fulva* (ex *Cladosporium fulvum*)**

3. Host species.....*Lycopersicum esculentum*
4. Source of inoculum .....Naktuinbouw (NL) or GEVES (FR)
5. Isolate .....Race group 0, A, B, C, D, and E
6. Establishment isolate identity.....with genetically defined differentials from GEVES (FR)
..... A breaks Cf-2, B Cf-4, C Cf-2&4, D Cf-5, E Cf-2&4&5
7. Establishment pathogenicity .....symptoms on susceptible tomato

8. Multiplication inoculum .....

8.1 Multiplication medium.....Potato Dextrose Agar or Malt Agar
8.8 Shelf life/viability inoculum.....4 hours, keep cool

9. Format of the test

9.1 Number of plants per genotype more than 20
9.3 Control varieties

Susceptible: .....Monalbo, Moneymaker
Resistant for race 0: ..... Angela, Estrella, Sonatine, Sonato, Vemone
Resistant for race group A: .....Angela, Estrella, Sonatine, Sonato
Resistant for race group B: .....Angela, Estrella, Sonatine, Sonato, Vemone
Resistant for race group C: .....Angela, Estrella, Sonatine
Resistant for race group D: .....Estrella, Sonatine, Vemone
Resistant for race group E: .....Sonatine

9.4 Test design.....2 plants per pot
9.5 Test facility.....glasshouse or climate room
9.6 Temperature .....day: 22° C, night: 20°
9.7 Light .....more than 12 hours
9.9 Special measures .....humidity tent closed 3 days after inoculation
.....After this, 66% closed during day, until end

10. Inoculation

10.1 Preparation inoculum .....prepare evenly colonized plates, 1 for 36 plants;
.....remove spores from plate by scraping 2-3 times with 1 ml
.....demi water with 0,01% Tween20;
.....filter through double muslin cloth
10.2 Quantification inoculum.....count spores; adjust to 5.10^5 spores per ml
10.3 Plant stage at inoculation.....19-20 d (incl. 12 d at 24°), 2-3 leaves
10.4 Inoculation method.....spray on dry leaves
10.7 End of test.....14 days after inoculation

11. Observations

11.1 Method .....visual inspection of abaxial side of inoculated leaves
11.2 Observation scale .....Symptom: velvety, white spots
11.3 Validation of test.....on standard varieties
11.4 Off-types.....excessively high humidity may cause
.....rugged brown spots on all leaves

12. Interpretation of data in terms of UPOV characteristic states

[1] symptoms
[9] no symptoms

13. Critical control points:

Ff spores have a variable size and morphology. Small spores are also viable.
Fungal plates will gradually become sterile after 6-10 weeks. Store good culture at -80°C.
For practical purposes, it is not possible to keep plants longer than 14 days inside a tent.

Literature references

Hubbeling, N., 1978. Breakdown of resistance to the Cf-5 gene in tomato by another new race of *Fulvia fulva*. Mededelingen van de Faculteit Landbouwwetenschappen Universiteit Gent 42/2

Laterrot, H., 1981. La lutte génétique contre la Cladosporiose de la Tomate en France, P.H.M. Revue Horticole, No. 214, February 1981.

Ad 51: Resistance to Tomato mosaic virus (ToMV)

1. Pathogen **Tomato mosaic virus**

3. Host species.....*Lycopersicum esculentum*
4. Source of inoculum .....Naktuinbouw (NL) or GEVES (F)
5. Isolate .....Strain 0, 1 and 2
6. Establishment isolate identity.....genetically defined tomato standards
.....Mobaci (Tm1) , Moperou (Tm2), Momor ($Tm2^2$)
7. Establishment pathogenicity .....on susceptible plant

8**. Multiplication inoculum**

8.1 Multiplication medium.....living plant
8.2 Multiplication variety.....Moneymaker, Marmande
8.7 Check of harvested inoculum...on *Nicotiana tabacum* "Xanthi", check lesions after 2 days
8.8 Shelf life/viability inoculum.....fresh>1 day, desiccated>1year

9. Format of the test

9.1 Number of plants per genotype at least 20
9.3 Control varieties

Susceptible.....Marmande, Monalbo
Resistant for ToMV: 0 and 2.....Mobaci
Resistant for ToMV: 0 and 1 .....Moperou
Resistant with necrosis....."Monalbo x Momor"
Resistant .....Gourmet

9.4 Test design.....blank treatment with PBS and carborundum
9.5 Test facility.....Glasshouse or climate room
9.6 Temperature .....25°C day, 23°C night
9.7 Light .....16 hours
9.8 Season.....symptoms are more pronounced in summer

10. Inoculation

10.1 Preparation inoculum .....1 g leaf with symptoms with 10 ml PBS
.....Homogenize, add carborundum to PBS (1 g/30ml)
10.3 Plant stage at inoculation.....2 leaves
10.4 Inoculation method.....gentle rubbing with sponge wetted with inoculum
10.5 First observation.....11 days after inoculation
10.7 End of test.....19 days after inoculation

11. Observations

11.1 Method .....visual
11.2 Observation scale .....Symptoms of susceptibility:
.....Mosaic in top, Leaf malformation
.....Symptoms of resistance (based on hypersensitivity):
.....Local Necrosis, Top necrosis, Systemic Necrosis

Remark: in some varieties a variable proportion of plants may have severe systemic necrosis or some necrotic spots while the other plants have no symptoms. This proportion may vary between experiments

12. Interpretation of data in terms of UPOV characteristic states

[1] symptoms of susceptibility
[9] no symptoms, or symptoms of hypersensitive resistance

13. Critical control points:

Temperature and light may influence the development of necrosis. More light means more necrosis. Resistant varieties may have symptomless plants and plants with severe necrosis; in spite of apparent segregation the sample may be evaluated as uniform for resistance

Remark ..... Strain INRA Avignon 6-5-1-1 is recommended. This strain
..... causes a striking yellow Aucuba mosaic

Literature references

Laterrot, H., 1973: Résistance de la Tomate au virus de la Mosaïque du Tabac. Difficultés rencontrées pour la sélection de variétés résistantes, Ann.Amelior.Plantes, 1973, 23(4), 287-313.

Ad 52: Resistance to *Phytophthora infestans* (Pi)

1. Pathogen ***Phytophthora infestans***
3. Host species.....*Lycopersicon esculentum*
4. Source of inoculum .....-
5. Isolate .....highly pathogenic on tomato
6. Establishment isolate identity.....biotest
7. Establishment pathogenicity .....biotest
8**. Multiplication inoculum**
8.1 Multiplication medium.....V8 agar
8.2 Multiplication variety.....Moneymaker
8.3 Plant stage at inoculation.....4 weeks
8.4 Inoculation medium.....water
8.5 Inoculation method.....spraying
8.6 Harvest of inoculum.....wash spores from wetted plates
8.7 Check of harvested inoculum...count sporangiospores
8.8 Shelf life/viability inoculum.....4 h after chilling at 8-10°C
9. Format of the test
9.1 Number of plants per genotype 20
9.3 Control varieties .....
Susceptible.....Saint Pierre, Heinz 1706
Resistant .....Pieraline, Heline, Pyros, "Pieraline x Pieralbo"
Remark: .....heterozygote varieties may have a slightly lower level of expression of resistance.
9.5 Test facility.....glasshouse
9.6 Temperature .....18°C
9.7 Light .....after inoculation darkness during 24 hours, thereafter 10 hour darkness per 24 hours
9.9 Special measures .....humidity tent during four days after inoculation
10. Inoculation
10.1 Preparation inoculum .....wash spores from sporulating leaves, chill at 8-10°C
.....Chilling will induce zoospore release
Remark .....Use fresh spores from repeated infection cycles on tomato plants
.....during 3 weeks before inoculation
10.2 Quantification inoculum.....count sporangiospores; adjust to 10^4 spores per ml
10.3 Plant stage at inoculation.....10 leaves developed (6 to 7 weeks)
10.4 Inoculation method.....spraying
10.5 First observation.....5 days after incoulation
10.6 Second observation ..... 6 days after inoculation
10.7 End of test.....7 days after inoculation
11. Observations
11.1 Method .....visual
11.2 Observation scale .....Symptoms: water-soaked lesions, yellowing, and death
11.3 Validation of test.....on standards
12. Interpretation of data in terms of UPOV characteristic states
.....[1] severe symptoms
.....[9] no or mild symptoms
13. Critical control points:.....resistance is only well-expressed in the adult plant

Literature references Laterrot, H., 1975: Sélection pour la résistance au Mildiou, Phytophthora infestans Mont. De Bary chez la Tomate, Ann.Amelior.Plantes, 1975, 25(2), 129-149.

Ad 53: Resistance to *Pyrenochaeta lycopersici* (Pl)

1. Pathogen ***Pyrenochaeta lycopersici***
3. Host species ..... *Lycopersicon esculentum*
4. Source of inoculum ..... -
5. Isolate ..... -
7. Establishment pathogenicity ..... biotest
8. Multiplication inoculum
8.1 Multiplication medium V8A
8.2 Multiplication variety ..... susceptible tomato variety
8.3 Plant stage at inoculation ..... seed
8.4 Inoculation medium ..... mixture of soil (70%), sand (20%) and inoculum (10.1) (10%)
..... or soil mixed with diseased roots cut to small pieces
8.5 Inoculation method ..... sowing
8.6 Harvest of inoculum ..... diseased roots are harvested after 2-4 months
8.7 Check of harvested inoculum ... visual inspection of lesions on roots
8.8 Shelf-life/viability inoculum .... the fungus will not die quickly, but may loose its pathogenicity within a week after isolation on an agar medium
9. Format of the test
9.1 Number of plants per genotype 20
9.3 Control varieties .....
susceptible: ..... Montfavet H 63.5
resistant: ..... Kyndia, Moboglan, Pyrella
9.5 Test facility ..... greenhouse or climate cell
9.6 Temperature ..... day 24°C, night 14°C
9.7 Light ..... 12 h minimum
.....
10. Inoculation
10.1 Preparation inoculum ..... double-autoclaved mixture of soil with 10% oatmeal added
..... Incubate for 10-14 d at 20°C with occasional, repeated turning
10.3 Plant stage at inoculation ..... 6 weeks
10.4 Inoculation method ..... transplanting into mixture of soil, sand and inoculum (8.4)
..... or soil mixed with diseased roots cut to small pieces
10.5 First observation ..... 6 weeks after transplanting
10.7 End of test ..... 8 weeks after transplanting (flowering plant)
11. Observations
11.1 Method ..... visual
11.2 Observation scale ..... Symptoms: brown lesions on roots
11.3 Validation of test ..... on standard varieties
12. Interpretation of data in terms of UPOV characteristic states
` [1] symptoms
[9] no symptoms
13. Critical control points:
The fungus looses its pathogenicity quickly after isolation on an agar medium. It is advisable to keep the isolate alive on living plants.

Literature references

Laterrot, H., 1983: La lutte génétique contre la maladie des racines liégeuses de la Tomate, P.H.M. Revue Horticole, No. 238, June-July 1983.

Ad 54: Resistance to *Stemphylium solani* (Ss)

1. Pathogen ***Stemphylium solani*** (see note below)
3. Host species.....*Lycopersicon esculentum*
4. Source of inoculum .....-
5. Isolate .....-
7. Establishment pathogenicity .....biotest
8. Multiplication inoculum
8.1 Multiplication medium.....PDA (12 hours per day under near-ultraviolet light
.....to induce sporulation)
9. Format of the test
9.1 Number of plants per genotype 20
9.3 Control varieties .....
Susceptible: .....Monalbo
Resistant:.....Motelle, F1 Motelle x Monalbo
99.5 Test facility.....greenhouse or climate cell
9.6 Temperature .....24°C
9.7 Light .....12 hours minimum
9.9 Special measures .....incubation in tunnel with 100 % relative humidity
10. Inoculation
10.1 Preparation inoculum .....sporulating plates (8.1) are scraped and air-dried overnight
.....The next day plates are soaked and strirred for 30 min
.....in a beaker with demineralized water.
..... The spore suspension is sieved through a double layer of muslin.
10.2 Quantification inoculum.....$5.10^3 – 10^5$ spores per ml
10.3 Plant stage at inoculation.....20-22 days (three expanded leaves)
10.4 Inoculation method.....spraying
10.5 First observation.....4 days after inoculation
10.6 Second observation .....5 days after inoculation
10.7 End of test.....6 days after inoculation
11. Observations
11.1 Method .....visual
11.2 Observation scale .....Symptoms:
.....necrotic lesions on cotyledons and leaves;
.....yellowing of leaves
11.3 Validation of test.....on standard varieties
12. Interpretation of data in terms of UPOV characteristic states
[1] symptoms (11.2)
[9] no symptoms, or less than resistant standard
13. Critical control points:.....8.1 and 10.1
Note: Some isolates of *Stemphylium* cannot be classified easily as either *Stemphylium solani* or a related species. These *Stemphylium* isolates may still be useful for identifying resistance to *Stemphylium solani*.

Literature references

Laterrot, H. and Blancard, D., 1983: Criblage d'une série de lignées et d'hybrides F1 de Tomate pour la résistance à la Stemphyliose, Phytopath. medit. 1983, 22, 188-193.

Laterrot, H. and Blancard, D., 1986: Les Stemphylia rencontrés sur la Tomate, Phytopath. medit. 1986, 25, 140-144.

Ad 55: Resistance to *Pseudomonas syringae* pv. *tomato* (Pst)

1. Pathogen ***Pseudomonas syringae* pv. *tomato* (see note below)**

3. Host species.....*Lycopersicon esculentum*
4. Source of inoculum .....GEVES or Naktuinbouw
5. Isolate .....
6. Establishment isolate identity.....
7. Establishment pathogenicity .....biotest

8. Multiplication inoculum

8.1 Multiplication medium.....King's B agar medium, darkness
8.2 Multiplication variety.....
8.3 Plant stage at inoculation.....
8.4 Inoculation medium.....water
8.5 Inoculation method.....
8.6 Harvest of inoculum.....
8.7 Check of harvested inoculum...
8.8 Shelf life/viability inoculum.....plates become old after 10 days

9. Format of the test

9.1 Number of plants per genotype 20
9.2 Number of replicates.....
9.3 Control varieties.....
Susceptible: .....Monalbo
Resistant: .....Ontario 7710, "Monalbo x Ontario 7710",
.....Tradiro, Hypeel 45
9.4 Test design.....
9.5 Test facility.....greenhouse in winter, growth chamber in summer
9.6 Temperature .....day: 22° C, night: 16° C
9.7 Light .....12 hours
9.8 Season.....
9.9 Special measures .....humidity tent needed for 3 days

10. Inoculation

10.1 Preparation inoculum .....wash off spores from plate. Plate should be less that 2-4 days old.
10.2 Quantification inoculum.....dilution plating, density 10^6 colony forming units per ml
10.3 Plant stage at inoculation.....three leaves expanded (20-22 days)
10.4 Inoculation method.....spraying a bacterial suspension on leaves
10.7 End of test..... 8 days after inoculation

11. Observations

11.1 Method .....visual
11.2 Observation scale ..... bacterial speck, greasy in appearance with marginal chlorosis
..... pinpoint lesions < 1.0 mm
11.3 Validation of test..... on standards
12. Interpretation of data in terms of UPOV characteristic states
.....[1] bacterial speck
.....[9] no symptoms or pinpoint lesions
13. Critical control points:.....Strains may lose virulence in storage

Option for testing without using the pathogen

Resistance to *Pseudomonas syringae* pv. *tomato* is often based on the *Pto* resistance gene. The presence of the *Pto* resistance gene may be detected without a biotest by spraying 10 µl/ml of the fungicide fenthion to small plants or to detached leaves (Martin et al 1994). This test should be performed on a minimum of 20 plants.

Literature references

Martin, G. B., A. Frary, T. Wu, S. Brommonschenkel, J. Chunwongse, E. D. Earle, S. D. Tanksley (1994) A member of the tomato Pto family confers sensitivity to fenthion resulting in rapid cell death. The Plant Cell 6: 1543-1552

Ad 56: Resistance to *Ralstonia solanacearum*, race 1 (Rs)

1. Pathogen ***Ralstonia solanacearum*** (ex *Pseudomonas solanacearum)*

3. Host species.....*Lycopersicon esculentum*

4. Source of inoculum .....

5. Isolate Race 1 has a wide host range, including tomato.
Race 3 has a narrow host range, also including tomato

8. Multiplication inoculum

8.1 Multiplication medium.....YPG

Special conditions: ..... 25-30°C (Race 3 usually needs 20-23°C)

8.5 Inoculation method.....2 ml of inoculum placed at the foot of each plantlet
.....prior to transplanting

8.8 Shelf life/viability inoculum.....suspension in sterile distilled water at 15°C (<1 year)

9. Format of the test.....

9.1 Number of plants per genotype 20

9.3 Control varieties

Susceptible: .....Floradel

Resistant: .....Caraibo

9.5 Test facility..... climate room

9.6 Temperature .....day: 26-30° C; night: 25° C

9.7 Light .....10 - 12 hours

9.9 Special measures .....high humidity

10. Inoculation

10.2 Quantification inoculum.....density 10^7 colony forming units per ml

10.3 Plant stage at inoculation.....three to four well-developed leaves (3 weeks)

10.4 Inoculation method.....

10.7 End of test.....

11. Observations

11.1 Method .....

11.2 Observation scale .....

11.3 Validation of test .....

12. Interpretation of data in terms of UPOV characteristic states

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

1. Pathogen **Tomato yellow leaf curl virus** (see note below)
2. Quarantine status ..... yes
3. Host species ..... *Lycopersicon esculentum*
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
9.3 Control varieties
Susceptible: ..... local varieties
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 End of test..... 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 ..... on standard varieties

12. Interpretation of data in terms of UPOV characteristic states
[1] severe symptoms
[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. Maintenance of TYLCV is only possible in living plants and *Bemisia* white flies. Transmission of TYLCV is only possible through *Bemisia* white flies. Mechanical transmission is not possible. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).\

Literature references

Barbieri, M., et al., 2010. Introgressions of resistance to two Mediterranean virus species causing tomato yellow leaf curl into a valuable traditional tomato variety. Journal of Plant Pathology 92(2):485-493

Ad 58: Resistance to Tomato spotted wilt virus (TSWV)

1. Pathogen **Tomato spotted wilt virus** (see note below)
2. Quarantine status ..... yes (see note below)
3. Host species ..... *Lycopersicon esculentum*
4. Source of inoculum ..... Naktuinbouw (NL)
5. Isolate ..... race 0, preferably a thrips-transmission deficient variant
7. Establishment pathogenicity ..... biotest

8. Multiplication inoculum

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
9.3 Control varieties
Susceptible: ..... Monalbo
Resistant: ..... Tsunami, Bodar, Lisboa
9.5 Test facility ..... glasshouse
9.6 Temperature ..... 20°C
9.7 Light ..... 16 hours
9.9 Special measures ..... prevent or combat thrips

10. Inoculation

10.1 Preparation inoculum ..... press symptomatic leaves in ice-cold buffer
..... 0,01 M PBS, pH 7.4, with 0,01 M sodium sulfite
..... sieve the leaf sap through double muslin
10.3 Plant stage at inoculation..... one or two expanded leaves
10.4 Inoculation method..... mechanical, rubbing with carborundum on cotyledons, inoculum suspension < 10° C
10.5 First observation ..... 7 days after inoculation
10.6 Second observation ..... 14 days after inoculation
10.7 End of test..... 21 days after inoculation

11. Observations

11.1 Method ..... visual
11.2 Observation scale ..... Symptoms: top mosaic, bronzing, various malformations, necrosis
11.3 Validation of test ..... on standard varieties

12. Interpretation of data in terms of UPOV characteristic states

[1] symptoms
[9] no symptoms

13. Critical control points:

TSWV has a quarantine status in some countries. TSWV is transmitted by *Thrips tabac*i and Western flower thrips (*Frankliniella occidentalis*). Pathotype 0 is defined by its inability to break resistance in tomato varieties carrying the resistance gene Sw-5. TSWV resistance based on Sw-5 may be detected without using the pathogen.

Note: Option for testing without using the pathogen

Resistance to TSWV:0 is often based on the resistance gen Sw-5. The presence of the resistance gene *Sw*-5 can be detected by molecular marker Sw-5b-LRR (Garland et al., 2005). This molecular test is validated to be used instead of a pathotest, as foreseen in UPOV document TC/38/14 Add. – CAJ/45/5 Add. under Option 1(a). Each molecular marker should be applied to a minimum of twenty plants and validated with proper controls.

Literature references

Garland, S., Sharman, M., Persley, D. and McGrath, D. (2005) The development of an improved PCR-based marker system for *Sw*-5, an important TSWV resistance gene of tomato. Australian Journal of Agricultural Research, 56 (3): 285-289.

Gordillo, L.F. and M. R. Stevens (2008) Screening two *Lycopersicon peruvianum* collections for resistance to Tomato spotted wilt virus. Plant Disease 92(5): 694-704

Smilde, W.D. and D. Peters (2007) Pathotyping TSWV in pepper and tomato. In: Niemorowicz-Szczytt, K. (Ed.), Progress in Research on Capsicum and Eggplant, Eucarpia conference proceedings, Warsaw, pp. 231-236

Ad 59: Resistance to *Leveillula taurica* (Lt)

1. Pathogen ***Leveillula taurica***

3. Host species.....*Lycopersicon esculentum*

4. Source of inoculum .....no long term storage method is available

5. Isolate .....

8.1 Multiplication medium.....detached leaves of a susceptible host plant

9. Format of the test

9.1 Number of plants per genotype 20

9.3 Control varieties .....

Susceptible: ..... Monalbo

Resistant: ..... Atlanta

10. Inoculation

10.1 Preparation inoculum .....

10.2 Quantification inoculum.....

10.3 Plant stage at inoculation.....adult plants

10.4 Inoculation method.....natural infection, mainly by wind dispersal of spores

10.7 End of test.....before harvest

11. Observations

11.1 Method .....visual

11.2 Observation scale .....Symptoms: Yellow chlorotic spots on upper side of leaves,
.....mycelium on abaxial side of leaves

Remark: .....Check cleistothecia under microscope to confirm presence of
.....*Leveillula* and not another powdery mildew.

11.3 Validation of test.....on standards

12. Interpretation of data in terms of UPOV characteristic states

Ad 60: Resistance to *Oidium neolycopersici* (Ol)

1. Pathogen ***Oidium neolycopersici* (Powdery mildew)**
3. Host species.....*Lycopersicon esculentum*
4. Source of inoculum .....-
5. Isolate see remark under 13
7. Establishment pathogenicity .....biotest

8. Multiplication inoculum
8.1 Multiplication medium.....plant
8.3 Plant stage at inoculation.....24°C during the day; 18°C during the night
8.4 Inoculation medium.....water
8.5 Inoculation method.....see 10.4
8.6 Harvest of inoculum.....by washing off
8.7 Check of harvested inoculum...check for contaminants under microscope
8.8 Shelf-life/viability inoculum ....1-2 hours

9. Format of the test
9.1 Number of plants per genotype 20
9.3 Control varieties .....
Susceptible: .....Momor
Resistant tomato: .....Atlanta
9.5 Test facility..... glasshouse
9.6 Temperature ..... 20°C
9.7 Light ..... 12 hours

10. Inoculation
10.1 Preparation inoculum .....collect spores in water
10.2 Quantification inoculum..... 10^4 conidia/ml
10.3 Plant stage at inoculation..... 3 weeks
10.4 Inoculation method..... by spraying on leaves or dredging of leaves
10.5 First observation.....7 days after inoculation
10.6 Second observation .....14 days after inoculation
10.7 End of test.....18 days after inoculation

11. Observations
11.1 Method .....visual
11.2 Observation scale .....0. no sporulation
.....1. necrotic points and sometimes locally restricted sporulation
.....2. moderate sporulation
.....3. abundant sporulation
11.3 Validation of test.....on standard varieties

12. Interpretation of data in terms of UPOV characteristic states
.....[1] Moderate or abundant sporulation
..... [9] No or restricted sporulation

13. Critical control points:
Resistance-breaking isolates should be avoided. Resistance to *O. neolycopersici* is usually race-specific. However, as long as a differential series of tomato genotypes with well defined resistances is lacking, it will remain hard to conclude that different races of *O. neolycopersici* exist.

Literature references

Bai, Y. 2004. The genetics and mechanisms of resistance to tomato powdery mildew (*Oidium neolycopersici*) in *Lycopersicon* species. Thesis Wageningen University, The Netherlands.

General literature references

Arens P., Mansilla C., Deinum D., Cavellini L., Moretti A., Rolland S., van der Schoot H., Calvache D., Ponz F., Collonnier C., Mathis R., Smilde D., Caranta C,; Vosman B., 2010. Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing. Theoretical and applied genetics. 120(3): 655-64

Kjellberg, L., 1973: Sortundersökningar av tomat enligt UPOV, Swedish University of Agricultural Sciences, Research Information Centre, Alnarp Trädgaard 162, SE.

Laterrot, H., 1990: Situation de la lutte génétique contre les parasites de la Tomate dans les pays méditerranéens, P.H.M. Revue Horticole, No. 303, January 1990.

Laterrot, H., 1982: L'argenture de la Tomate, P.H.M. Revue Horticole, No. 225, March 1982.

Webreference

http://www.worldseed.org/isf/pathogen_coding_3.html (International Seed Federation (ISF), Trade Issues, Phytosanitary Matters, Pathogen coding, Strain Denomination, Differential sets)

[End of Annex II and of document]