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| --- | --- | --- |
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| INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS | | |
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Technical working party for vegetables

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Partial Revision of the Test Guidelines for Cucumber  
(document TG/61/7)

Document prepared by experts from the Netherlands

The purpose of this document is to present the proposals for the partial revision of the Test Guidelines for Cucumber (document TG/61/7).

The following changes are proposed:

1. Revision of grouping characteristics, including the behavior against pathogens
2. a revised format for disease resistance characteristics according to the explanations for disease resistance characteristics in Test Guidelines and eventual new proposal to update the protocol
   * Chapter 5: Grouping of Varieties and Organization of the Growing Trial
   * Chapter 7: Table of Characteristics
   * Chapter 8: Explanations on the Table of Characteristics
     + 8.2 Explanations for individual characteristics
   * Chapter 10 : Technical Questionnaire
     + Paragraph 5: Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).

The proposed revisions are presented in the Annex to this document.

[Annex follows]

Proposal for a Revision of the Grouping Characteristics in Chapter 5.3

*Current wording:*

1. Cotyledon: bitterness (characteristic 1)
2. Plant: sex expression (characteristic 13)
3. Ovary: color of vestiture (characteristic 15)

(c) Parthenocarpy (characteristic 16)

(d) Fruit: length (characteristic 17)

(e) Fruit: ground color of skin at market stage (characteristic 25)

*Proposed new wording:*

1. Cotyledon: bitterness (characteristic 1)
2. Plant: sex expression (characteristic 13)
3. Ovary: color of vestiture (characteristic 15)

(c) Parthenocarpy (characteristic 16)

(d) Fruit: length (characteristic 17)

(e) Fruit: ground color of skin at market stage (characteristic 25)

(f) Resistance to *Cladosporiumcucumerinum* (Ccu) (characteristic 44)

(g) Resistance to *Cucumber mosaic virus* (CMV) (characteristic 45)

(h) Resistance to powdery mildew (*Podosphaeraxanthii*) (Sf) (characteristic 46)

(i) Resistance to Corynespora blight and target leaf spot (*Corynesporacassiicola*) (Cca) (characteristic 48)

(j) Resistance to *Cucumber vein yellowing virus* (CVYV) (characteristic 49)

Proposal to revise Characteristics 44 to 47

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 44.   (+) |  | Resistance to *Cladosporium cucumerinum* (Ccu) | Résistance à *Cladosporium cucumerinum* (Ccu) | Resistenz gegen *Cladosporium cucumerinum* (Ccu) | Resistencia a la *Cladosporium cucumerinum* (Ccu) |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Pepinex 69 | 1 |
|  |  | present | présente | vorhanden | presente | Maketmore 76 | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 44.  (\*) (+) |  | Resistance to *Cladosporium cucumerinum* (Ccu) | Résistance à *Cladosporium cucumerinum*(Ccu) | Resistenz gegen *Cladosporium cucumerinum* (Ccu) | Resistencia a la *Cladosporium cucumerinum* (Ccu) |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Pepinex 69 | 1 |
|  |  | present | présente | vorhanden | presente | Marketmore 76 | 9 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 45.   (+) |  | Resistance to Cucumis Mosaic Virus (CMV) | Résistance au virus de la mosaïque du concombre | Resistenz gegen Gurkenmosaikvirus (CMV) | Resistencia al virus del mosaico del pepino (CMV) |  |  |
| **QN** |  | susceptible | sensibilité | anfällig | susceptible | Gele Tros | 1 |
|  |  | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Gardon | 2 |
|  |  | highly resistant | forte résistance | hochresistent | alta | Hokus, Naf | 3 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 45.  (\*) (+) |  | Resistance to *Cucumber mosaic virus* (CMV) | Résistance au virus de la mosaïque du concombre (CMV) | Resistenz gegen Gurkenmosaikvirus (CMV) | Resistencia al virus del mosaico del pepino (CMV) |  |  |
| **QN** |  | susceptible | Sensibilité | anfällig | Susceptible | Ventura | 1 |
|  |  | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Gardon, Verdon | 2 |
|  |  | highly resistant | forte résistance | hochresistent | alta | Naf, Picolino | 3 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 46.   (+) |  | Resistance to powdery mildew (*Podosphaera xanthii*) (Sf) | Résistance à l’oïdium (*Podosphaera xanthii*) (Sf) | Resistenz gegen Echten Mehltau (*Podosphaera xanthii*) (Sf) | Resistencia al mildiú blanco (*Podosphaera xanthii*) (Sf) |  |  |
| **QN** |  | susceptible | sensibilité | anfällig | susceptible | Corona | 1 |
|  |  | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Flamingo | 2 |
|  |  | highly resistant | forte résistance | hochresistent | alta | Cordoba | 3 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 46.  (\*) (+) |  | Resistance to Powdery mildew (*Podosphaera xanthii*) (Px) | Résistance à l’oïdium (*Podosphaera xanthii*) (Px) | Resistenz gegen Echten Mehltau (*Podosphaera xanthii*) (Px) | Resistencia al mildiú blanco (*Podosphaera xanthii*) (Px) |  |  |
| **QN** |  | susceptible | sensibilité | anfällig | susceptible | Corona | 1 |
|  |  | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Flamingo | 2 |
|  |  | highly resistant | forte résistance | hochresistent | alta | Cordoba | 3 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 47.   (+) |  | Resistance to downy mildew (*Pseudoperonospora cubensis*) (Pc) | Résistance au mildiou (*Pseudoperonospora cubensis*) (Pc) | Resistenz gegen Falschen Mehltau (*Pseudoperonospora cubensis*) (Pc) | Resistencia al mildiú velloso del pepino (*Pseudoperonospora cubensis* (Pc)) |  |  |
| **QN** |  | susceptible | sensibilité | anfällig | susceptible | Pepinex 69, SMR 58 | 1 |
|  |  | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Poinsett | 2 |
|  |  | highly resistant | forte résistance | hochresistent | alta |  | 3 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 47.   (+) |  | Resistance to Downy mildew (*Pseudoperonospora cubensis*) (Pc) | Résistance au mildiou (*Pseudoperonospora cubensis*) (Pc) | Resistenz gegen Falschen Mehltau (*Pseudoperonospora cubensis*) (Pc) | Resistencia al mildiú velloso del pepino (*Pseudoperonospora cubensis* (Pc)) |  |  |
| **QN** |  | susceptible | sensibilité | anfällig | susceptible | Pepinex 69, SMR 58 | 1 |
|  |  | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Poinsett 76 | 2 |
|  |  | highly resistant | forte résistance | hochresistent | alta |  | 3 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 48.   (+) |  | Resistance to Corynespora blight and target leaf spot (*Corynespora cassiicola*) (Cca) | Résistance à la pourriture corynespora et à la septoriose (*Corynespora cassiicola*) (Cca) | Resistenz gegen Corynespora-Blattfleckenkrank-heit (*Corynespora cassiicola*) (Cca) | Resistencia a la mancha foliar (*Corynespora cassiicola*) (Cca) |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Cerrucho, Goya, Pepinova | 1 |
|  |  | present | présente | vorhanden | presente | Corona, Cumlaude, Edona | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 48. (\*) (+) |  | Resistance to Corynespora blight and target leaf spot (*Corynespora cassiicola*) (Cca) | Résistance à la pourriture corynespora et à la septoriose (*Corynespora cassiicola*) (Cca) | Resistenz gegen Corynespora-Blattfleckenkrankheit (*Corynespora cassiicola*) (Cca) | Resistencia a la mancha foliar (*Corynespora cassiicola*) (Cca) |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Bodega | 1 |
|  |  | present | présente | vorhanden | presente | Corona, Cumlaude | 9 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 49.   (+) |  | Resistance to Cucumber Vein Yellowing Virus (CVYV) | Résistance au virus du jaunissement des nervures du concombre | Resistenz gegen Cucumber Vein Yellowing Virus (CVYV) | Resistencia al virus de las venas amarillas del pepino (CVYV) |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Corona | 1 |
|  |  | present | présente | vorhanden | presente | Tornac | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 49.  (\*) (+) |  | Resistance to *Cucumber vein yellowing virus* (CVYV) | Résistance au virus du jaunissement des nervures du concombre (CVYV) | Resistenz gegen *Cucumber vein yellowing virus* (CVYV) | Resistencia al virus de las venas amarillas del pepino (CVYV) |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Corona | 1 |
|  |  | present | présente | vorhanden | presente | Tornac | 9 |

*Current wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 50.   (+) |  | Resistance to Zucchini Yellow Mosaic Virus (ZYMV) | Résistance au virus de la mosaïque jaune de la courgette | Resistenz gegen Zucchinigelb-mosaikvirus (ZYMV) | Resistencia al virus del mosaico amarillo del calabacín (ZYMV) |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Corona | 1 |
|  |  | present | présente | vorhanden | presente | Dina | 9 |

*Proposed new wording:*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 50.   (+) |  | Resistance to *Zucchini yellow mosaic virus* (ZYMV) | Résistance au virus de la mosaïque jaune de la courgette (ZYMV) | Resistenz gegen Zucchinigelb-mosaikvirus (ZYMV) | Resistencia al virus del mosaico amarillo del calabacín (ZYMV) |  |  |
| **QL** |  | absent | absente | fehlend | ausente | Corona | 1 |
|  |  | present | présente | vorhanden | presente | Dina | 9 |

Proposal to Include a Revised Format for Disease Resistance Characteristics

(Current and Proposed New Wording are presented on opposite pages)

*Current wording:*

Ad. 44: Resistance to *Cladosporium cucumerinum* (Ccu)

Method

Maintenance of disease

|  |  |  |
| --- | --- | --- |
|  | Type of medium: | PDA (Potato Dextrose Agar) |
|  | Special conditions: | 7-8 days in the dark at 20°C |
|  | Remarks: | The spore suspension should have a concentration of 0.5 x 105 spores/ml. To be kept for a maximum of 4 days in a refrigerator at 4°C. |

|  |  |
| --- | --- |
| Preparation of inoculum: | Scrape off the fungus from the PDA medium, collect in a beaker and filter through a cheese‑cloth. |

Raising the plants

|  |  |  |
| --- | --- | --- |
|  | Sowing: | In potting soil or compost |
|  | Temperature: | 22/20°C (day/night) |
|  | Light: | At least 16 hours |
|  | Number of plants: | 30 plants per sample |

Inoculation

|  |  |  |
| --- | --- | --- |
|  | Growth stage of plants: | The plants should have a first leaf with a diameter of 3 cm. |
|  | Method of inoculation: | Spray spore suspension on leaves |

Special conditions after inoculation

|  |  |  |
| --- | --- | --- |
|  | Temperature: | 22/20°C (day/night) |
|  | Light: | At least 16 hours |
|  | Special conditions: | Plastic cover placed over the plants. The plastic cover is closed during the first three days and thereafter slightly opened during the daytime. |

Duration of test

|  |  |  |
| --- | --- | --- |
|  | - From sowing to inoculation: | 12 days |
|  | - From inoculation to last reading: | 6-8 days |

|  |  |
| --- | --- |
| Standard varieties: | Resistance absent: Pepinex 69 |
|  | Resistance present: Maketmore 76 |

*Proposed new wording:*

Ad. 44: Resistance to *Cladosporium cucumerinum* (Ccu)

|  |  |
| --- | --- |
| 1. Pathogen | *Cladosporium cucumerinum* |
| 2. Quarantine status | No |
| 3. Host species | *Cucumissativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw (NL) |
| 5. Isolate |  |
| 6. Establishment isolate identity | expected reactions on resistant standard varieties |
| 7. Establishment pathogenicity | symptoms on susceptible standard varieties |
| 8. Multiplication inoculum |  |
| 8.1 Multiplication medium | Agar medium e.g.:Potato Dextrose Agar (PDA) |
| 8.2 Multiplication variety |  |
| 8.3 Plant stage at inoculation |  |
| 8.4 Inoculation medium | sterile demineralized water |
| 8.5 Inoculation method | scrape the Petri dishes and spread over new plates |
| 8.6 Harvest of inoculum | from 7-8 days old subcultures in the dark at 20°C |
| 8.7 Check of harvested inoculum |  |
| 8.8 Shelflife/viability inoculum | 4 days at 4°C |
| 9. Format of the test |  |
| 9.1 Number of plants per genotype | at least 20 |
| 9.2 Number of replicates |  |
| 9.3 Control varieties | Frontera, Cherubino, Pepinex 69 (Susceptible)  Corona, Sheila, Marketmore 76 (Resistant) |
| 9.4 Test design | e.g. after every 8 samples 16 resistant and 16 susceptible plants |
| 9.5 Test facility |  |
| 9.6 Temperature | 18 or 22/20°C d/n |
| 9.7 Light | at least 16 hours |
| 9.8 Season | Best results obtained in February-April due to temperature |
| 9.9 Special measures | Make sure soil is not dry at time of inoculation; plastic tent closed day and night during first three days after inoculation; thereafter slightly opened during daytime |
| 10. Inoculation |  |
| 10.1 Preparation inoculum | optional: add 0,01% Tween to spore suspension |
| 10.2 Quantification inoculum | 0.5\*105 -0.5\*106 spores/mL |
| 10.3 Plant stage at inoculation | young cotyledon or first true leaf |
| 10.4 Inoculation method | spraying spore suspension |
| 10.5 First observation | 6 dpi |
| 10.6 Second observation | 8 dpi |
| 10.7 Final observations | 8 dpi |
| 11. Observations |  |
| 11.1 Method | Visual, comparative |
| 11.2 Observation scale |  |
| [1] Susceptible: Frontera | brown lesions on cotyledons and plant death |
| [9] Resistant: Corona | without symptoms, or with green lesions, or browning of the leaves |
| 11.3 Validation of test | on standards |
| 11.4 Off-types | maximum 1 of 6-35 plants |
| 12. Interpretation of data in terms of  UPOV characteristic states | QL |
| 13. Critical control points | temperature and humidity |

*Current wording:*

Ad.  45: Resistance to Cucumis Mosaic Virus (CMV)

Method

Maintenance of disease

|  |  |  |
| --- | --- | --- |
|  | Type of medium: | On susceptible living plants |
|  | Remarks: | Greenhouse to be kept free from aphids |

|  |  |
| --- | --- |
| Preparation of inoculum: | Mix freshly infected leaves with water. Prepare a solution with a concentration of 1:15 (inoculum: water). |

Raising the plants

|  |  |  |
| --- | --- | --- |
|  | Sowing: | In potting soil or compost |
|  | Temperature: | 22/20°C (day/night) |
|  | Light: | At least 16 hours |
|  | Number of plants: | 30 plants per sample |

Inoculation

|  |  |  |
| --- | --- | --- |
|  | Growth stage of plants: | Fully developed cotyledons |
|  | Method of inoculation: | Mechanical inoculation, by rubbing the cotyledons using carborundum powder. Carborundum powder to be washed away after inoculation. |

Special conditions after inoculation

|  |  |  |
| --- | --- | --- |
|  | Temperature: | 22/20°C (day/night) |
|  | Light: | 16 hours |

Duration of test

|  |  |  |
| --- | --- | --- |
|  | - From sowing to inoculation: | 6-7 days |
|  | - From inoculation to last reading: | 10-14 days |

|  |  |  |
| --- | --- | --- |
| Scheme of observation: |  |  |
|  |  |  |
| 1. Susceptible |  |  |
|  |  |  |
| II | restricted growth, cotyledon slightly blistered, leaves completely mottled | Gele Tros |
| III | curled leaves, heavy mosaic symptoms over whole leaf |  |
|  |  |  |
| 2. Moderately resistant |  |  |
|  |  |  |
| IV | curled leaves, slight mosaic symptoms | Gardon |
| V | slightly curled leaves, slight mosaic symptoms, many necrotic spots |  |
| VI | leaves not curled, vague mosaic symptoms, few necrotic spots |  |
| 3. Highly resistant |  |  |
|  |  |  |
| VII | very few virus symptoms, very few necrotic spots |  |
| VIII | no symptoms | Hokus, Naf |

*Proposed new wording:*

Ad.  45: Resistance to *Cucumber mosaic virus* (CMV)

|  |  |
| --- | --- |
| 1. Pathogen | *Cucumber mosaic virus* |
| 2. Quarantine status | No |
| 3. Host species | *Cucumissativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw (NL), GEVES (FR) |
| 5. Isolate | e.g.UK 6 |
| 6. Establishment isolate identity | resistant and susceptible controls or ELISA dipstick (Agdia) |
| 7. Establishment pathogenicity | susceptible control inoculation |
| 8. Multiplication inoculum |  |
| 8.1 Multiplication medium | on susceptible living plants |
| 8.2 Multiplication variety | susceptible control |
| 8.3 Plant stage at inoculation | cotyledons |
| 8.4 Inoculation medium | ice-cold Phosphate Buffer Solution +carborundum+ active charcoal |
| 8.5 Inoculation method | Rubbing |
| 8.6 Harvest of inoculum | fresh symptomatic leaf |
| 8.7 Check of harvested inoculum | mock inoculation with PBS + carborundum |
| 8.8 Shelflife/viability inoculum | 8 h at 4°C or on ice |
| 9. Format of the test |  |
| 9.1 Number of plants per genotype | at least 30 |
| 9.2 Number of replicates | 3 |
| 9.3 Control varieties | Corona, Ventura, Bosporus (Susceptible), Gardon, Verdon, Capra (Intermediate Resistant), Picolino, Naf |
| 9.4 Test design | e.g. replicates on different tablets in glasshouse |
| 9.5 Test facility | glasshouse or climatic chamber |
| 9.6 Temperature | 18-25°C /15-20°C d/n or 22°C constant |
| 9.7 Light | at least 16h |
| 9.8 Season | Best results in Apr/May; Sep/Oct |
| 9.9 Special measures | keep glasshouse free of aphids |
| 10. Inoculation |  |
| 10.1 Preparation inoculum | fresh leaf ground in cold PBS |
| 10.2 Quantification inoculum |  |
| 10.3 Plant stage at inoculation | Cotyledons, e.g.:8 and 11 d after sowing |
| 10.4 Inoculation method | rubbing, rinse carborundum off |
| 10.5 First observation | 7 dpi |
| 10.6 Second observation | 14 dpi |
| 10.7 Final observations | 21 dpi, first and second leaf symptoms;  only needed when second observation is not decisive |
| 11. Observations |  |
| 11.1 Method | visual estimate of mosaic severity on 1st leaf |
| 11.2 Observation scale |  |
| [1] Susceptible: 3, Ventura, Corona | mosaic; clear border between yellow and green |
| [1] Susceptible: 4, Bosporus | heavy mottle; confluent chlorosis |
| [2] Moderately resistant: 5, Verdon,  Gardon | light mottle; chlorotic islands |
| [2] Moderately resistant: 6, Capra | some chlorotic stippling |
| [3] Highly resistant: 7, Picolino, Naf | no symptoms |
| 11.3 Validation of test | Standards should conform to description; describe if different  Variation within standard should not exceed 1 scale point |
| 11.4 Off-types | 2 scale points difference with majority type |
| 12. Interpretation of data in terms of  UPOV characteristic states | QN; [1] 3-4; [2] 5-6; [3] 7 |
| 13. Critical control points | 1. Symptoms will develop from ring spot into mosaic (Ventura) or mottle (Gardon) or spots (Capra) Observation should focus on mature symptoms.  2. Aphids may transmit CMV as well as other viruses that may contaminate the CMV strain. Test should be in aphid-free compartment  3. Growth inhibition is usually not strong enough to measure in young plants; severe growth inhibition is more likely caused by genetic aberration than by virus infection  4. Leaf curling is not mentioned as a CMV symptom because leaf curling is usually caused by unbalanced growing conditions.  5. Replicates are intended to control the main source of variation. For CMV this is usually the amount of sunlight. Therefore, replicate tablets should represent the different levels of shading within one greenhouse compartment. |

*Current wording:*

Ad. 46: Resistance to powdery mildew *(Podosphaera xanthii)* (Sf)

Method

Maintenance of disease

Type of medium: On susceptible living plants

Preparation of inoculum: Wash the spores from the infected leaves and prepare a suspension with a concentration of 105 spores/ml. Filter the suspension through a cheese-cloth before infecting the plants.

Raising the plants

Sowing: In potting soil or compost

Temperature: 22/20°C (day/night)

Light: At least 16 hours

Number of plants: 30 plants per sample

Inoculation

Growth stage of plants: Fully developed cotyledons

Method of inoculation: Spray spore suspension on leaves on the first, second and fifth day after planting out.

Special conditions after inoculation

Temperature: 20/20°C (day/night)

Light: 16 hours

Duration of test

- From sowing to inoculation: 7, 8 and 11 days

- From inoculation to last reading: 12 days

Scheme of observation

1. Susceptible: hypocotyls and cotyledons infected, first leaf strongly infected, high sporulation.

2. Moderately resistant: hypocotyls not infected, cotyledons and first leaf moderately infected with moderate sporulation, moderate colonization.

3. Highly resistant: hypocotyls and cotyledons not infected, first leaf very weakly or not infected, few colonies, very weak sporulation.

Standard varieties: 1. Susceptible: Corona

2. Moderately resistant: Flamingo

3. Highly resistant: Cordoba

*Proposed new wording:*

Ad. 46: Resistance to Powdery mildew *(Podosphaera xanthii)* (Px)

|  |  |
| --- | --- |
| 1. Pathogen | Powdery mildew*Podosphaera xanthii* (*Sphaerotheca fuliginea)* |
| 2. Quarantine status | No |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | natural or Naktuinbouw (NL) |
| 5. Isolate |  |
| 6. Establishment isolate identity | expected reactions on resistant standard varieties |
| 7. Establishment pathogenicity | symptoms on susceptible standard varieties |
| 8. Multiplication inoculum |  |
| 8.1 Multiplication medium | Plants |
| 8.2 Multiplication variety | susceptible variety (e.g. Ventura) |
| 8.3 Plant stage at inoculation | first leaf appearing |
| 8.4 Inoculation medium | Demineral water |
| 8.5 Inoculation method | Spraying |
| 8.6 Harvest of inoculum | Wash spores off from sporulating leaves with demineralized water,  Option: add Tween20 at 5 µL (1 drop) /liter  filter with cheese-cloth. 0,75 ml/pl |
| 8.7 Check of harvested inoculum | Count spores; target concentration is 1.105 spores/ml |
| 8.8 Shelflife/viability inoculum | 15 minutes |
| 9. Format of the test |  |
| 9.1 Number of plants per genotype | at least 20 |
| 9.2 Number of replicates |  |
| 9.3 Control varieties | Ventura, Corona (Susceptible) Flamingo (IR);  Bella, Aramon, Cordoba  (Resistant) |
| 9.4 Test design |  |
| 9.5 Test facility |  |
| 9.6 Temperature | 20°C constant |
| 9.7 Light | 16 h |
| 9.8 Season | Best results in autumn (Sep/Nov) |
| 9.9 Special measures |  |
| 10. Inoculation |  |
| 10.1 Preparation inoculum | as above at 8.6 |
| 10.2 Quantification inoculum | 1.105 spores/ml |
| 10.3 Plant stage at inoculation | cotyledon at 1st inoculation, first leaf at final inoculation |
| 10.4 Inoculation method | Spraying,  inoculation repeated on day 3, 5 and 6 after 1st |
| 10.5 First observation | 10 dpi |
| 10.6 Second observation | 14 dpi |
| 10.7 Final observations |  |
| 11. Observations |  |
| 11.1 Method | Visual, comparative; mainly on first leaf |
| 11.2 Observation scale | Sporulation on cotyledons and hypocotyls; heavy sporulation on first leaf |
| [1] Susceptible: Ventura, Corona | Sporulation on cotyledons and hypocotyls; heavy sporulation on first leaf |
| [2] Moderately resistant: Flamingo | No sporulation on hypocotyls,  Moderate sporulation on cotyledons and the first leaf; |
| [3] Highly resistant: Bella, Aramon,  Cordoba: | Symptoms on cotyledons are disregarded.  Sometimes very light sporulation on first leaf. |
| 11.3 Validation of test | on standard varieties |
| 11.4 Off-types | No more than 1 of 6-35 plants |
| 12. Interpretation of data in terms of  UPOV characteristic states | QN [1] susceptible; [2] intermediate; [3] resistant |
| 13. Critical control points | Some types of intermediate resistance may break down at higher temperatures |

*Current wording:*

Ad. 47: Resistance to downy mildew (*Pseudoperonospora cubensis*) (Pc)

Method

Maintenance of disease

Type of medium: On susceptible living plants

Preparation of inoculum: Wash the spores from the infected leaves with cold distilled water and prepare a suspension. Suspension to be used immediately.

Raising the plants

Sowing: In potting soil or compost

Temperature: 22/20°C (day/night)

Light: At least 16 hours

Number of plants: 30 plants per sample

Inoculation

Growth stage of plants: First two leaves fully developed

Method of inoculation: Spray spore suspension on leaves.

Special conditions after inoculation

Temperature: 22/20°C (day/night)

Light: 16 hours

Relative humidity: 100%, 48 hours after inoculation

Special conditions: Plastic cover placed over the plants. The plastic cover is closed during the first three days and thereafter slightly opened during the daytime.

Duration of test

- From sowing to inoculation: 20 days

- From inoculation to last reading: + 10 days

Scheme of observations:

Susceptible: Large lesions with abundant spore production, leaf tissue becoming necrotic within 5 days.

Moderately resistant: Medium lesions, period of tissue yellowing prolonged to beyond 10 days.

Highly resistant: Small downy mildew lesions, round tissue in the center becoming necrotic, no visual spore production.

Standard varieties: Susceptible: Pepinex 69, SMR 58

Moderately resistant: Poinsett

Highly resistant:

*Proposed new wording:*

Ad. 47: Resistance to Downy mildew (*Pseudoperonospora cubensis*) (Pc)

|  |  |
| --- | --- |
| 1. Pathogen | Downy mildew (*Pseudoperonospora cubensis*) |
| 2. Quarantine status | No |
| 3. Host species | *Cucumissativus* (cucumber or gherkin) |
| 4. Source of inoculum |  |
| 5. Isolate |  |
| 6. Establishment isolate identity | expected reactions on resistant standard varieties |
| 7. Establishment pathogenicity | symptoms on susceptible standard varieties |
| 8. Multiplication inoculum |  |
| 8.1 Multiplication medium | Living plants |
| 8.2 Multiplication variety | susceptible variety |
| 8.3 Plant stage at inoculation | two leaves |
| 8.4 Inoculation medium | Cold distilled water |
| 8.5 Inoculation method | Spraying |
| 8.6 Harvest of inoculum | By washing a sporulating leaf |
| 8.7 Check of harvested inoculum | By counting the spores |
| 8.8 Shelflife/viability inoculum |  |
| 9. Format of the test |  |
| 9.1 Number of plants per genotype | at least 20 |
| 9.2 Number of replicates |  |
| 9.3 Control varieties | Pepinex 69, SMR 58 (susceptible), Poinsett 76(intermediate resistant) |
| 9.4 Test design |  |
| 9.5 Test facility |  |
| 9.6 Temperature | 22/20°C d/n |
| 9.7 Light | at least 16h |
| 9.8 Season |  |
| 9.9 Special measures | keep 100% humidity for 24 h. A plastic cover is placed over the plants. After 24 h, the plastic cover is slightly opened during daytime. |
| 10. Inoculation |  |
| 10.1 Preparation inoculum | By washing sporulating leaves |
| 10.2 Quantification inoculum | Counting spores 103 spores per ml |
| 10.3 Plant stage at inoculation | first two leaves fully developed |
| 10.4 Inoculation method | by spraying spore suspension on leaves |
| 10.5 First observation | 7 dpi |
| 10.6 Second observation | 10 dpi |
| 10.7 Final observations |  |
| 11. Observations |  |
| 11.1 Method | Visual, comparative |
| 11.2 Observation scale |  |
| [1] Susceptible: Pepinex 69, SMR58 | large lesions with abundant sporulation, leaf tissue becoming necrotic within 5 days |
| [9] Moderately resistant: Poinsett76 | small circular lesions, necrotic in the center, sporulation visible macroscopically no highly resistant standard is available |
| [9] Highly resistant: | On standards |
| 11.3 Validation of test |  |
| 11.4 Off-types |  |
| 12. Interpretation of data in terms of  UPOV characteristic states | QL [1] susceptible; [9] moderately and highly resistant |
| 13. Critical control points |  |

*Current wording:*

Ad. 48: Resistance to Corynespora blight and target leaf spot (*Corynespora cassiicola*) (Cca)

Method

Maintenance of disease

Type of medium: PDA (Potato Dextrose Agar)

Special conditions: 12-14 days in the dark at 20°C

Remarks: The spore suspension should have a concentration of 0.5 x 105 spores/ml. To be kept for a maximum of 4 days in a refrigerator at 4°C

Preparation of inoculum: Scrape off the fungus from the nutrient medium, collect in a beaker and filter through a cheese‑cloth.

Raising the plants

Sowing: In potting soil or compost

Temperature: 22/20°C (day/night)

Light: At least 16 hours

Number of plants: 30 plants per sample

Inoculation

Growth stage of plants: The plants should have a first leaf with a diameter of 3 cm.

Method of inoculation: Spray spore suspension on leaves

Special conditions after inoculation

Temperature: 25/15°C (day/night)

Light: At least 16 hours

Special conditions: Plastic cover placed over the plants. The plastic cover is closed during the first three days and thereafter slightly opened during the daytime.

Duration of test

- From sowing to inoculation: 12-13 days

- From inoculation to last reading: 8-10 days

Scheme of observation:

1. Susceptible

a. cotyledons and first leaf dead, plant with greatly reduced growth

b. cotyledons dead or strongly infected, first leaf weakly infected, plant with greatly reduced growth

2. Resistant

a. cotyledons heavily infected, first leaf not infected, plant with normal growth

b. cotyledons and first leaf not infected, plant with normal growth

Standard varieties:

Susceptible: Pepinova (1a) and Cerrucho, Goya (1b)

Resistant: Cumlaude, Edona (2a) and Corona (2b)

*Proposed new wording:*

Ad. 48: Resistance to Corynespora blight and target leaf spot (*Corynespora cassiicola*) (Cca)

|  |  |
| --- | --- |
| 1. Pathogen | *Corynespora cassiicola* (Target leaf spot) |
| 2. Quarantine status | No |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw (NL) |
| 5. Isolate |  |
| 6. Establishment isolate identity | expected reactions on resistant standard varieties |
| 7. Establishment pathogenicity | symptoms on susceptible standard varieties |
| 8. Multiplication inoculum |  |
| 8.1 Multiplication medium | PDA at 20°C in darkness |
| 8.2 Multiplication variety |  |
| 8.3 Plant stage at inoculation |  |
| 8.4 Inoculation medium | demineralized water |
| 8.5 Inoculation method | scraping the Petri dishes and spread over new plates |
| 8.6 Harvest of inoculum | from 12-14 d old subcultures |
| 8.7 Check of harvested inoculum |  |
| 8.8 Shelflife/viability inoculum | max. 4 d at 4°C |
| 9. Format of the test |  |
| 9.1 Number of plants per genotype | at least 20 |
| 9.2 Number of replicates |  |
| 9.3 Control varieties | Pepinova, Cerrucho, Goya (Susceptible); Corona, Cumlaude, Edona (Resistant) |
| 9.4 Test design |  |
| 9.5 Test facility |  |
| 9.6 Temperature | 25/15°C d/n or 23°Cd/n in climatic chamber |
| 9.7 Light | at least 16h |
| 9.8 Season | Best results obtained in February-April due to temperature |
| 9.9 Special measures | Make sure soil is not dry at time of inoculation; plastic tent closed day and night 3 dpi, closed only in night >3 dpi |
| 10. Inoculation |  |
| 10.1 Preparation inoculum | Filter through cheesecloth; add 0,01% Tween to spore suspension |
| 10.2 Quantification inoculum | 0,5x105 spores/ml |
| 10.3 Plant stage at inoculation | diameter first true leaf around 3 cm  transplant on day 7, then inoculate on day 12 |
| 10.4 Inoculation method | spraying spore suspension |
| 10.5 First observation | 8-11 days |
| 10.6 Second observation |  |
| 10.7 Final observations |  |
| 11. Observations |  |
| 11.1 Method | Visual; comparative; mainly on cotyledon and first leaf |
| 11.2 Observation scale |  |
| [1] 1, Highly susceptible: Bodega | Cotyledons dead, first leaves dead, growth retardation |
| [1] 2, Susceptible: Cerrucho | Cotyledons dead or covered with lesions, first leaves with lesions, growth retardation |
| [9] 3, Resistant: Cumlaude | Cotyledons with a few lesions, first leaf with no or sometimes a few lesions |
| [9] 4, Highly resistant: Corona | Cotyledons without lesions; first leaf without lesions |
| 11.3 Validation of test | Standards should conform to description; describe if different |
| 11.4 Off-types | maximum 1 of 6-35 plants |
| 12. Interpretation of data in terms of  UPOV characteristic states | QL |
| 13. Critical control points |  |

*Current wording:*

Ad. 49: Resistance to Cucumber Vein Yellowing Virus (CVYV)

Method

Maintenance of isolate

Type of medium: On susceptible living plants

Special conditions: Fresh inoculum, or inoculum which has been stored for a maximum of 3 months at ‑20˚C

Execution of test

Growth stage of plants: Appearance of first leaf

Temperature: 16 to 30˚C

Light: 16 hours

Growing method: Greenhouse

Method of inoculation: Mechanical, by rubbing of cotyledons

Duration of test: From inoculation to reading: 14 days

Number of plants tested: At least 15 plants

Standard varieties: Susceptible: Corona

Resistant: Tornac

Remark: Resistant varieties may have a slight discoloration of the veins of older leaves

*Proposed new wording:*

Ad. 49: Resistance to *Cucumber vein yellowing virus* (CVYV)

|  |  |
| --- | --- |
| 1. Pathogen | *Cucumber vein yellowing virus* |
| 2. Quarantine status | No |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw |
| 5. Isolate | e.g. KB18 |
| 6. Establishment isolate identity | resistant and susceptible controls |
| 7. Establishment pathogenicity | susceptible control inoculation |
| 8. Multiplication inoculum |  |
| 8.1 Multiplication medium | leaf |
| 8.2 Multiplication variety | susceptible variety (e.g. Korinda) |
| 8.3 Plant stage at inoculation | cotyledons / appearance of first leaf |
| 8.4 Inoculation medium | Leaf in ice-cold PBS + carborundum |
| 8.5 Inoculation method | Rubbing |
| 8.6 Harvest of inoculum | freeze-dried leaf |
| 8.7 Check of harvested inoculum |  |
| 8.8 Shelflife/viability inoculum | 8 h at 4°C or on ice |
| 9. Format of the test |  |
| 9.1 Number of plants per genotype | at least 30 |
| 9.2 Number of replicates |  |
| 9.3 Control varieties | Corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) |
| 9.4 Test design |  |
| 9.5 Test facility | greenhouse |
| 9.6 Temperature | 16-30°C |
| 9.7 Light | 16h at least |
| 9.8 Season | Best results in Apr/May; Sep/Oct |
| 9.9 Special measures | 12.000 lux suggested; keep glasshouse free of aphids |
| 10. Inoculation |  |
| 10.1 Preparation inoculum | fresh leaf ground in 0.03 M phosphate buffer + carborundum + active charcoal |
| 10.2 Quantification inoculum |  |
| 10.3 Plant stage at inoculation | cotyledons |
| 10.4 Inoculation method | rubbing, Option: rinse carborundum off to prevent leaf damage |
| 10.5 First observation | 7 dpi; cotyledon symptoms |
| 10.6 Second observation | 14 dpi; first leaf symptoms |
| 10.7 Final observations | 21 dpi, first and second leaf symptoms |
| 11. Observations |  |
| 11.1 Method | Visual; comparative; mainly on first leaf |
| 11.2 Observation scale |  |
| [1] Susceptible 3. Korinda, Corona | mosaic; clear border between yellow and green |
| [1] Susceptible 4. Ventura | heavy mottle; confluent chlorosis |
| [9] Resistant 5. Dina | light mottle; chlorotic islands |
| [9] Resistant 6. Summerstar | some chlorotic stippling |
| [9] Resistant 7. Tornac | no symptoms |
| 11.3 Validation of test | Standards should conform to description; describe if different. Variation within standard should not exceed 1 scale point |
| 11.4 Off-types |  |
| 12. Interpretation of data in terms of  UPOV characteristic states | QL; [1] 3-4, Susceptible; [9] 5-7, Resistant |
| 13. Critical control points | resistant varieties may have a slight discoloration of the veins of older leaves |

*Current wording:*

Ad. 50: Resistance to Zucchini Yellow Mosaic Virus (ZYMV)

Method

Maintenance of isolate

Type of medium: On susceptible living plants

Special conditions: Fresh inoculum, or inoculum which has been stored for a maximum of 6 months at - 20˚C

Execution of test

Growth stage of plants: Appearance of first leaf

Temperature: 23 to 25˚C day and night

Light: 16 hours

Growing method: Greenhouse

Method of inoculation: Mechanical, by rubbing of cotyledons

Duration of test: From inoculation to reading: 14 days

Number of plants tested: At least 15 plants

Standard varieties: Susceptible: Corona

Resistant: Dina

Remark: Resistant varieties may have a slight discoloration of the veins of older leaves.

Susceptible varieties have systemic mosaic symptoms.

*Proposed new wording:*

Ad. 50: Resistance to *Zucchini yellow mosaic virus* (ZYMV)

|  |  |
| --- | --- |
| 1. Pathogen | *Zucchini yellow mosaic virus* |
| 2. Quarantine status | No |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw |
| 5. Isolate | e.g. CU61 |
| 6. Establishment isolate identity | resistant and susceptible controls; |
| 7. Establishment pathogenicity | susceptible control inoculation |
| 8. Multiplication inoculum |  |
| 8.1 Multiplication medium | Leaf |
| 8.2 Multiplication variety | susceptible control |
| 8.3 Plant stage at inoculation | cotyledons / appearance of first leaf |
| 8.4 Inoculation medium | ice-cold PBS + carborundum |
| 8.5 Inoculation method | rubbing |
| 8.6 Harvest of inoculum | fresh or dried leaf |
| 8.7 Check of harvested inoculum |  |
| 8.8 Shelflife/viability inoculum | 8 h at 4°C or on ice |
| 9. Format of the test |  |
| 9.1 Number of plants per genotype | at least 30 |
| 9.2 Number of replicates |  |
| 9.3 Control varieties | Corona, Ventura, Hilton (Susceptible), Thunder, Summerstar, Dina (Resistant) |
| 9.4 Test design |  |
| 9.5 Test facility | greenhouse or climatic chamber |
| 9.6 Temperature | 18-25°C /15-25°C d/n |
| 9.7 Light | at least 16h |
| 9.8 Season | best results in Apr/May; Sep/Oct |
| 9.9 Special measures | 12.000 lux suggested; keep glasshouse free of aphids |
| 10. Inoculation |  |
| 10.1 Preparation inoculum | fresh leaf ground in cold PBS |
| 10.2 Quantification inoculum |  |
| 10.3 Plant stage at inoculation | cotyledons / appearance of first leaf -(e.g. 8d.; repeat 3 d later) |
| 10.4 Inoculation method | rubbing, rinsecarborundum off |
| 10.5 First observation | 7 - 14 dpi; cotyledon symptoms |
| 10.6 Second observation | 14 - 21 dpi; first leaf symptoms |
| 10.7 Final observations | 21 dpi, first and second leaf symptoms |
| 11. Observations |  |
| 11.1 Method | Visual. Comparative, mainly on first leaf |
| 11.2 Observation scale |  |
| [1] Susceptible: 4. Ventura, Corona | Mosaic; leaf deformation |
| [1] Susceptible: 5. Hilton | Mosaic; weak leaf deformation |
| [9] Resistant: 6. Thunder | Weak mottle |
| [9] Resistant: 7. Summerstar, Dina | Vein necrosis |
| 11.3 Validation of test | Standards should conform to description; describe if different.  Variation within standard should not exceed 1 scale point |
| 11.4 Off-types | 2 scale points difference with majority type |
| 12. Interpretation of data in terms of  UPOV characteristic states | QL: [1]4-5: Susceptible; [9] 6-7: Resistant |
| 13. Critical control points | Resistant varieties may have a slight discoloration of the veins of older leaves. Susceptible varieties have systemic mosaic symptoms. |

Proposed changes to Chapter 10 “Technical Questionnaire”

To add the following characteristics to Chapter TQ 5:

Resistance to *Cladosporiumcucumerinum* (Ccu) (characteristic 44)

Resistance to *Cucumber mosaic virus* (CMV) (characteristic 45)

Resistance to powdery mildew (*Podosphaeraxanthii*) (Sf) (characteristic 46)

Resistance to Corynespora blight and target leaf spot (*Corynesporacassiicola*) (Cca) (characteristic 48)

Resistance to *Cucumber vein yellowing virus* (CVYV) (characteristic 49)

[End of Annex and of document]