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| International Union for the Protection of New Varieties of Plants |  |

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| Technical Committee  Fifty-Eighth Session Geneva, October 24 and 25, 2022 | TC/58/25  Original: English  Date: October 5, 2022 |

MATTERS TO BE RESOLVED CONCERNING TEST GUIDELINES PUT FORWARD FOR ADOPTION BY THE TECHNICAL COMMITTEE: TOMATO ROOTSTOCKS

Document prepared by an expert from the Netherlands

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The Enlarged Editorial Committee (TC-EDC), at its meeting held in Geneva, October 25 to 26, 2021, considered a proposal for a partial revision of the Test Guidelines for Tomato Rootstocks (document TC/57/18). The TC-EDC agreed that the technical issues raised on the proposed partial revision should be addressed by the Technical Working Party for Vegetables (TWV) (see document TC/57/25 “Report”, Annex II).

The TWV, at its fifty-sixth session[[1]](#footnote-2), considered document TWV/56/8 “Matters to be resolved concerning Test Guidelines put forward for adoption by the Technical Committee: Tomato Rootstocks” including the technical issues identified by the TC-EDC (indicated below by “#”). The proposed responses by the Leading Expert, Ms. Cécile Marchenay (Netherlands), and the conclusions of the TWV were as follows (see document TWV/56/2 “Report”, paragraph 81:

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| #Char. 22,  Ad. 22 | to check whether to reduce the scale to 3 notes or to improve the explanation about scoring the characteristic using all notes on the scale of 5 notes.  *Leading Expert: Scale 1 to 5 has been kept for the moment, including extra explanation about all notes.*  *TWV:*   |  |  | | --- | --- | | *Char. 22* | *to read as follows:* |  |  |  |  |  |  | | --- | --- | --- | --- | --- | | 22.  (\*) (+) | VG | Resistance to *Meloidogyne incognita* (Mi) |  |  | | **QN** |  | susceptible | Bruce | 1 | |  |  | intermediate resistant |  | 2 | |  |  | highly resistant | Emperador | 3 |  |  |  | | --- | --- | | *Ad. 22, 4.* | *reference to INIA to read “INIA – CSIC”* | | *Ad. 22, 8.5* | *to read “deposit of piece of inoculated roots…”* | | *Ad. 22, 9.1* | *to add "It is recommended to include in the test, 10 non-inoculated plants, to be able to identified a possible lack of germination or a delay in plant growth, due to the material."* | | *Ad. 22, 10.2* | *to read "The aggressiveness of the test depends on the quantity of inoculum and the growing conditions (e.g. between 30g to 60g of inoculated roots "* | | *Ad. 22, 10.4* | *to read “Seeds are sown in non-inoculated soil and inoculation of soil…”* | | *Ad. 22, 12.* | *to read*  *“[1] Susceptible: variety very similar to susceptible control*  *[2] Intermediate resistant: variety very similar to intermediate resistant control*  *[3] Highly resistant: variety very similar to highly resistant control*  *If results are not clear, statistical analysis is advised.*  *If significantly different from the controls, a retest is advised to check if the result is stable.”* | |
| #Ad. 22, 9.1, 9.4, 11.3 | to improve the explanation clarifying how would germination effect the scoring of the characteristic  *Leading Expert: see Annex to this document*  *TWV: agreed* |
| Ad. 22, 9.1 | to read “… due to nematode ~~or not~~”  *Leading expert: see Annex to this document (covered by comment above)*  *TWV: agreed* |
| Ad. 22, 9.2 | to read “at least 2, preferably 3 ~~to allow statistical analysis~~”  *Leading Expert: prefer to keep “to allow statistical analysis”*  *TWV: agreed* |
| Ad. 22, 9.6 | to read “20-26°C, the temperature ~~may~~ should be …”  *Leading Expert: agreed*  *TWV: agreed* |
| Ad. 22, 10.2 | to read “~~the ratio is depending of~~ Quantity of inoculum depends on aggressiveness of test and ~~lab’s~~ growing conditions (e.g. between 30 g to 60 g of infested roots~~,~~ for 100 plants in a tray of 45\*30 cm containing approximately 5.5 kg of substrate);~~,~~ galls should be homogeneously mixed with soil.”  *Leading Expert: agreed*  *TWV: agreed* |
| Ad. 22, 10.4 | to read “~~plants~~ seed sown in soil contaminated with galls.”  *Leading Expert: agreed*  *TWV: agreed* |
| Ad. 22, 11.4 | to be deleted  *Leading Expert: agreed*  *TWV: agreed* |
| Ad. 22, 12. | in the figure, blue text: “Tyonoc” should read “Tyonic”  *Leading Expert: figure removed, not applicable*  *TWV: agreed* |
| Ad. 24, 12. | - to add the following wording:  “Absent [1] distribution of plants in the classes comparable with the susceptible controls.  “Present [9] distribution of plants in the classes comparable with the resistant controls.”  *Leading Expert: agreed*  *TWV: agreed* |

The Annex to this document presents a new proposal for the explanation Ad. 22, based on the information above.

Changes previously agreed to the Test Guidelines of Tomato Rootstocks, presented in document TC/57/18, will be incorporated in the final adopted version of the partial revision of the Test Guidelines for Tomato Rootstocks.

[Annex follows]

Proposed changes to the explanation Ad. 22 “Resistance to *Meloidogyne incognita* (Mi)”

Ad. 22: Resistance to *Meloidogyne incognita* (Mi)

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| 1. | Pathogen | *Meloidogyne incognita* |
| 2. | Quarantine status | - |
| 3. | Host species | Tomato - *Solanum lycopersicum* |
| 4. | Source of inoculum | GEVES[[2]](#footnote-3) (FR) or INIA – CSIC (ES)[[3]](#footnote-4) or Naktuinbouw (NL[[4]](#footnote-5)) |
| 5. | Isolate | non-resistance breaking |
| 6. | Establishment isolate identity | use tomato standards |
| 7 | Establishment pathogenicity | use susceptible rootstock or tomato standard |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | living plant |
| 8.2 | Multiplication variety | susceptible variety, preferably resistant to powdery mildew |
| 8.3 | Plant stage at inoculation | ~~see 10.3~~ 2nd leaf stage |
| 8.5 | Inoculation method | ~~see 10.4~~  deposit of piece of inoculated roots in soil (around 5-10g near each plant, to adapt depending on the population aggressivity) |
| 8.6 | Harvest of inoculum | 6 to 10 weeks after inoculation, 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 and ripe egg masses |
| 8.8 | Shelf life/viability inoculum | 1 day |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | ~~20 plants~~ 30 plants  Remark: knowing that germination in rootstocks might be low and/or irregular it is recommended to sow more seeds to be sure to get at least 30 plants.  It is recommended to include in the test, 10 non-inoculated plants, to be able to identify a possible lack of germination or a delay in plant growth, due to the material. |
| 9.2 | Number of replicates | ~~1 replicate~~  at least 2, preferably 3 to allow statistical analysis |
| 9.3 | Control varieties | Susceptible: Bruce and (*Solanum lycopersicum*) ~~Clairvil,~~ Casaque Rouge  ~~Moderately~~ Intermediate resistant: (*Solanum lycopersicum*)  ~~Madyta,~~ Campeon, ~~Madyta, Vinchy~~, Tyonic  Highly resistant: Emperador ~~and (~~*~~Solanum lycopersicum~~*~~) “Anahu x Casaque Rouge”, Anahu, Anabel~~ |
| 9.4 | Test design | ~~include standard varieties~~  3 replicates of 10 plants in different trays by variety~~, non-inoculated plants in a separate tray~~ |
| 9.5 | Test facility | greenhouse or climate room |
| 9.6 | Temperature | ~~not over 28° C~~  20-26°C, the temperature should be adapted, depending on the aggressiveness of the test, to obtain the expected response of the controls, but should not exceed 26°C. Higher temperatures will cause breakdown of resistance. |
| 9.7 | Light | at least 12 h per day |
| 10 | Inoculation |  |
| 10.1 | Preparation inoculum | small pieces of diseased roots mixed with soil  ~~mix soil and infested root pieces~~ |
| 10.2 | Quantification inoculum | ~~soil: root ratio = 8:1, or depending on experience~~  Quantity of inoculum depends on aggressivness of test and growing conditions (e.g. between 30g to 60g of inoculated roots for 100 plants in a tray of 45\*30 cm containing approximately 5.5 kg of substrate); galls should be homogeneously mixed with soil. |
| 10.3 | Plant stage at inoculation | seed~~, or cotyledons~~ |
| 10.4 | Inoculation method | ~~plants are sown in infested soil or contamination of soil after sowing when plantlets are at cotyledon stage~~  Seeds are sown in non-inoculated soil and inoculation of soil and inoculation of soil is done after sowing when plantlets are at cotyledon stage. |
| 10.7 | End of test | 28 to 45 days after inoculation depending on test conditions (temperature, season) |
| 11. | Observations |  |
| 11.1 | Method | root inspection per plant |
| 11.2 | Observation scale |  |
|  | | |
| 11.3 | Validation of test | ~~evaluation of variety resistance should be calibrated with results of resistant and susceptible controls on standards~~  Validation on controls. Expected reactions of controls:  Susceptible control: most plants at classes 3 and 4.  Highly resistant: most plants at classes 0 and 1.  Intermediate resistant: clearly different from other controls with majority of plants around class 2. |
| 11.4 | Off-types | resistant varieties may have a few plants with a few galls |
| 12. | Interpretation of ~~test results in comparison with control varieties~~ data in terms of UPOV characteristic states | [1] Susceptible: variety very similar to susceptible control  [2] Intermediate resistant: variety very similar to intermediate resistant control  [3] Highly resistant: variety very similar to highly resistant control  If results are not clear, statistical analysis is advised.  If significantly different from the controls, a retest is advised to check if the result is stable. |
|  | ~~To consider that resistant varieties may have a few plants with falls. These are not considered as off-types.~~  ~~absent (susceptible)………… [1] growth strongly reduced, high gall count~~  ~~intermediate   (moderately resistant)………… [2] medium growth reduction, medium gall count~~  ~~present (highly resistant)……… [3] no growth reduction, no galls~~ | |
| 13. | Critical control points | ~~Avoid rotting of roots; high temperature causes breakdown of resistance~~  Avoid overwatering. This may result in rotting of roots.  In case of aggressive test, decrease the quantity of inoculum. |

[End of Annex and of document]

1. organized by electronic means, from April 18 to 22, 2022 [↑](#footnote-ref-2)
2. GEVES; [matref@geves.fr](mailto:matref@geves.fr) [↑](#footnote-ref-3)
3. INIA; [resistencias@inia.es](mailto:resistencias@inia.es) [↑](#footnote-ref-4)
4. Naktuinbouw; [resistentie@naktuinbouw.nl](mailto:resistentie@naktuinbouw.nl) [↑](#footnote-ref-5)