UPOV
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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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1. 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 TWV (see document TC/57/25 "Report", Annex II).

2. The following table presents comments made by the TC-EDC on the proposed partial revision of the Test Guidelines for Tomato Rootstocks (document TC/57/18). The technical issues to be addressed by the TWV are indicated with "#". The proposed responses from the Leading Expert, Ms. Cécile Marchenay (Netherlands), are presented under each comment from the TC-EDC.

#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.*
#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*
Ad. 22, 9.1	to read "… due to nematode ~~or not~~" *Leading expert: see Annex to this document (covered by comment above)*
Ad. 22, 9.2	to read "at least 2, preferably 3 ~~to allow statistical analysis~~" *Leading Expert: prefer to keep "to allow statistical analysis"*
Ad. 22, 9.6	to read "20-26°C, the temperature ~~may~~ should be …" *Leading Expert: 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*
Ad. 22, 10.4	to read "~~plants~~ seed sown in soil contaminated with galls." *Leading Expert: agreed*
Ad. 22, 11.4	to be deleted *Leading Expert: agreed*
Ad. 22, 12.	in the figure, blue text: "Tyonoc" should read "Tyonic" *Leading Expert: figure removed, not applicable*
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*

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

[Annex follows]

ANNEX

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

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

1.	Pathogen	*Meloidogyne incognita*
2.	Quarantine status	-
3.	Host species	Tomato - *Solanum lycopersicum*
4.	Source of inoculum	GEVES[1] (FR) or INIA (ES)[2] or Naktuinbouw (NL[3])
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 contaminated 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.
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 aggressivity of test and growing conditions (e.g. between 30g to 60g 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.
10.3	Plant stage at inoculation	seed~~, or cotyledons~~

[1] GEVES; matref@geves.fr

[2] INIA; resistencias@inia.es

[3] Naktuinbouw; resistentie@naktuinbouw.nl

10.4	Inoculation method	~~plants are sown in infested soil or contamination of soil after sowing when plantlets are at cotyledon stage~~ Plants are sown in non-contaminated soil and contamination 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	

Class 0: healthy plant, no galls	Class 1: few and little galls which are difficult to find (for example less than 5)	Class 2: few galls, easy to observe but on few roots, still a lot of roots without galls	Class 3: many individual galls on most but not all roots	Class 4: many galls on all roots, sometimes in chains, can lead to dead plants and /or may suppress emergence
				

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: distribution of plants in the classes comparable with the susceptible controls. [2] Susceptible to intermediate resistant: distribution of plants in the classes between susceptible controls and intermediate resistant controls (significantly different from both). [3] Intermediate resistant: distribution of plants in the classes comparable with the intermediate resistant controls. [4] Intermediate resistant to highly resistant: distribution of plants in the classes between intermediate resistant controls and highly resistant controls (significantly different from both). [5] Highly resistant: distribution of plants in the classes comparable with the highly resistant controls. If results are not clear, statistical analysis is advised.
	~~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]