Fifty-Ninth Session Virtual meeting, May 5 to 8, 2025 Original: English Date: April 11, 2025

PARTIAL REVISION OF THE TEST GUIDELINES FOR BROCCOLI

Document prepared by an expert from the Netherlands (Kingdom of)

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1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Broccoli (document TG/151/5 Rev.).

2. The Technical Working Party for Vegetables (TWV), at its fifty-eighth session¹, agreed that the Test Guidelines for Broccoli (*Brassica oleracea* L. var. *italica* Plenck) be partially revised (see document TWV/58/11 "Report", Annex II).

- 3. The following changes are proposed:
 - (a) Addition of Characteristic 26 "Resistance to *Plasmodiophora brassicae* (Pb)", including example varieties.
 - (b) Addition of explanation Ad. 26 "Resistance to *Plasmodiophora brassicae* (Pb)"

4. The proposed changes are presented below in highlight and <u>underline</u> (insertion) and strikethrough (deletion).

¹ held via electronic means, from April 22 to 25, 2024.

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Proposed addition of Characteristic 26 "Resistance to *Plasmodiophora brassicae* (Pb)", including example varieties

26 (+)	VS	Resistance to Plasmodiophora brassicae (Pb)	Résistance à Plasmodiophora brassicae (Pb)	Resistenz gegen Plasmodiophora brassicae (Pb)	Resistencia a Plasmodiophora brassicae (Pb)		
26.1	VS	– Race Pb: 0	– Pathotype Pb: 0	– Pathotyp Pb: 0	– Raza Pb: 0		
QL		absent	absente	fehlend	ausente	Marathon	1
		present	présente	vorhanden	presente	Monclano	9
26.2	vs	– Race Pb: 1	– Pathotype Pb: 1	– Pathotyp Pb: 1	– Raza Pb: 1		
QL		absent	absente	fehlend	ausente	Marathon	1
		present	présente	vorhanden	presente	Monclano	9
26.3	vs	– Race Pb: 2	– Pathotype Pb: 2	– Pathotyp Pb: 2	– Raza Pb: 2		
QL		absent	absente	fehlend	ausente	Marathon, Monclano	1
		present	présente	vorhanden	presente		9
26.4	vs	– Race Pb: 3	– Pathotype Pb: 3	– Pathotyp Pb: 3	– Raza Pb: 3		
QL		absent	absente	fehlend	ausente	Marathon	1
		present	présente	vorhanden	presente	Monclano	9

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Proposed addition of an explanation Ad. 26 "Resistance to *Plasmodiophora brassicae* (Pb)" in Chapter 8.2 "Explanations for individual characteristics"

Ad. 26: Resistance to Plasmodiophora brassicae (Pb)

1.	Pathogen	Plasmodiophora brassicae	
2.	Quarantine status	no	
3.	Host species	Brassica oleracea	
4.	Source of inoculum	Naktuinbouw ² (NL)	
5.	Isolate	Race Pb: 0, Pb: 1, Pb: 2 and Pb: 3	
6.	Establishment isolate identity	with genetically defined differentials from Naktuinbouw (NL) The most recent table is available through ISF at https://www.worldseed.org/our-work/plant-health/differential-hosts/	
7.	Establishment pathogenicity	symptoms on susceptible Brassica oleracea spp.	
8.	Multiplication inoculum		
8.1	Multiplication medium	Plant roots	
8.2	Multiplication variety	Susceptible variety Bartolo (WC), Granaat (CC) ³	
8.3	Plant stage at inoculation	Seedling, 1 week after sowing	
8.4	Inoculation medium	Water	
8.5	Inoculation method	2 ml spore suspension (10 ⁷ sp/ml) Pipette to the base of each seedling.	
8.6	Harvest of inoculum	Harvest roots 6-8 weeks after inoculation	
8.7	Check of harvested Microscopic count inoculum		
8.8	Shelf life/viability inoculum	Frozen 3 years, room temperature 1-2 days	
9.	Format of the test		
9.1	Number of plants per genotype	20 plants	
9.2	Number of replicates	2 replicates (2 x 10)	
9.3	Control varieties	Susceptible: Bartolo (WC) ² Resistant to race Pb: 0 051632 Bejo (WC), Clapton (CF), Lodero (RC) Resistant to race Pb: 1 Clapton (CF), Lodero (RC) Resistant to race Pb: 2 Lodero (RC) Resistant to race Pb: 3 051632 Bejo (WC)	
9.5	Test facility	Glasshouse or climatic room	
9.6	Temperature	20-22°C	
9.7	Light	Natural, extended to 16 h if needed	
9.9	Special measures	A moderate amount of water is required to prevent rotting. Keep the soil saturated in the first week. During plant growth the soil should not be too dry to lower the soil temperature.	
9.8	Season	Not in winter, not in too warm conditions if test performed in greenhouse	
10.	Inoculation		
10.1	Preparation inoculum	Symptomatic roots are homogenized ca. 1 min in a blender. Dilute clubs 1:4 with demineralized water. Blender the mix for less than 1 minute. (Beware: longer blendering may cause overheating of the suspension)	
10.2	Quantification inoculum	count spores; adjust to 10 ⁷ spores per ml	
10.3	Plant stage at inoculation	1 week old seedlings	
10.4	Inoculation method	Pipette 1 ml on both sides at the base of each seedling, totalling 2 ml per plant.	

² Naktuinbouw: <u>resistentie@naktuinbouw.nl</u>

 $^{\rm 3}$ WC=White cabbage, CC=Chinese cabbage, RC=Red cabbage, CF=Cauliflower

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10.7	Observation, evaluation and end of test	6 weeks after inoculation (destructive)	
11.	Observations		
11.1	Method	Visual: observation of severe galling and growth retardation Destructive: observation on a 0-3 scale for galling	
11.2	Observation scale	class 0 = no swellings or a few small spheroid galls class 1 = very slight swelling, usually confined to the lateral roots class 2 = moderate swelling on lateral and/or tap roots or slight swelling of the main root and browning and ultimately death of all the lateral roots class 3 = severe swelling on lateral and/or tap roots	
11.3	Validation of test	Validation on controls. Expected response of controls: Susceptible control: -most plants in classes 2 and 3 Resistant control: -most plants in classes 0 and 1	
12.	Interpretation of data in terms of UPOV characteristic states	[1] absent: distribution of plants in the classes comparable with susceptible control[9] present: distribution of plants in the classes comparable with resistant control	
13.	Critical control points	Clubroot is a zoosporic pathogen. Keep isolates spatially well-separated.	







1 = a few small galls







2 = moderate galling 2 = slight swelling of the main 3 = severe galling root, no lateral roots

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