

Technical Working Party for Vegetables

TWV/57/21

Fifty-Seventh Session
Antalya, Türkiye, May 1 to 5, 2023

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PARTIAL REVISION OF THE TEST GUIDELINES FOR KOHLRABI

Document prepared by an expert from the Netherlands

Disclaimer: this document does not represent UPOV policies or guidance

1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Kohlrabi (document TG/65/4 Rev.).
2. The Technical Working Party for Vegetables (TWV) at its fifty-sixth session¹, agreed that the Test Guidelines for Kohlrabi (*Brassica oleracea* L. convar. *acephala* (DC.) Alef. var. *gongylodes* L. (*Brassica oleracea* L. *Gongylodes* Group)) be partially revised (see document TWV/56/22 "Report", Annex II).
3. The following changes are proposed:
 - (a) Revision of Characteristic 24 "Male sterility"
 - (b) Revision of explanation Ad. 24 "Male sterility"
 - (c) Addition of new Characteristics 25 to 28 "Resistance to *Plasmodiophora brassicae* (Pb)" Races Pb: 0, 1, 2 and 3 (clubroot)
 - (d) Addition of new explanation Ad. 25 to 28 "Resistance to *Plasmodiophora brassicae* (Pb)" Races Pb: 0, 1, 2 and 3
4. The proposed changes are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

¹ organized by electronic means, from April 18 to 22, 2022

Proposed revision of Characteristic 24 “Male sterility”*Current wording*

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
24. VG/ (*) (+)	Male sterility	Stérilité mâle	Männliche Sterilität	Androesterilidad		
QL²	absent	absente	fehlend	ausente	Expreß Forcer, Lanro	1
	present	présente	vorhanden	presente	Erika, Morre, Oasis	9

¹ See document TGP/7 “Development of Test Guidelines”, Annex 3 “Guidance Notes (GN) for the TG Template”, GN 25 “Recommendations for conducting the examination” (http://www.upov.int/edocs/tgpdocs/en/tgp_7.pdf)

² See document TGP/7 “Development of Test Guidelines”, Annex 3 “Guidance Notes (GN) for the TG Template”, GN 20 “Presentation of characteristics: States of expression according to type of expression of a characteristic”, 2. “Qualitative Characteristics” (http://www.upov.int/edocs/tgpdocs/en/tgp_7.pdf)

Proposed new wording

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
24. VG/ (*) (+)	Male sterility	Stérilité mâle	Männliche Sterilität	Androesterilidad		
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² See document TGP/7 “Development of Test Guidelines”, Annex 3 “Guidance Notes (GN) for the TG Template”, GN 20 “Presentation of characteristics: States of expression according to type of expression of a characteristic”, 2. “Qualitative Characteristics” (http://www.upov.int/edocs/tgpdocs/en/tgp_7.pdf)

Proposed revision of explanation Ad. 24 “Male sterility”*Current wording*Ad. 24: Male sterility

To be tested in a field trial and/or in a DNA marker test.

Field trial:

Check presence of pollen on stamen: if pollen on stamen is present then male sterility is absent; if pollen on stamen is absent then male sterility is present.

DNA marker test and/or field trial:

All varieties declared male sterile in the TQ can be examined in a field trial or in a DNA marker test³. In the case of a DNA marker test, if the CMS marker appears to be not present, a field trial should be performed to observe whether the variety is male sterile (on another mechanism) or fertile. All varieties declared fertile are to be tested in a field trial.

In case of a field trial, type of observation is VG. In case of a DNA marker test, type of observation is MS.

*Proposed new wording*Ad. 24: Male sterility

To be tested in a field trial and/or in a DNA marker test.

Field trial:

Check presence of pollen on stamen: if pollen on stamen is present then male sterility is absent; if pollen on stamen is absent then male sterility is present.

DNA marker test and/or field trial:

All varieties, whether declared male fertile or male sterile in the TQ, can be examined in a field trial or in a DNA marker test².

In the case of a DNA marker test, if the CMS marker appears to be not present, a field trial should be performed to observe whether the variety is male sterile (on another mechanism) or fertile. the variety is expected to have male fertile flowers. In cases where the CMS marker appears to be present, the variety is expected to have male sterile flowers. All varieties declared fertile are to be tested in a field trial.

In case the DNA marker test result does not confirm the declaration in the TQ, a field trial should be performed to observe whether the variety has male fertile or male sterile flowers due to another mechanism.

In the case of a field trial, the type of observation is VG VS. In the case of a DNA marker test, the type of observation is MS.

² The description of the method to test male sterility for *Brassica* (CMS marker) is covered by a trade secret. The owner of the trade secret, Syngenta Seeds B.V., has given its consent for the use of the CMS marker solely for the purposes of examination of Distinctness, Uniformity and Stability (DUS) and for the development of variety descriptions by UPOV and authorities of UPOV members. Syngenta Seeds B.V. declares that neither UPOV, nor authorities of UPOV members that use the CMS marker for the above purposes will be held accountable for possible (mis)use of the CMS marker by third parties. Please contact Naktuinbouw, Netherlands, to obtain the method and information on the CMS marker for the purposes mentioned above.

Proposed addition of new Characteristics 25 to 28 "Resistance to *Plasmodiophora brassicae* (Pb)" Races Pb: 0, 1, 2 and 3 (clubroot)

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
25.	VG	<u>Resistance to</u>	<u>Résistance à</u>	<u>Resistenz gegen</u>	<u>Resistencia a</u>	
(+)	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>		
	<u>- Race Pb: 0</u>	<u>- Pathotype Pb: 0</u>	<u>- Pathotyp Pb: 0</u>	<u>- Raza Pb: 0</u>		
QL	<u>absent</u>	<u>absente</u>	<u>fehlend</u>	<u>ausente</u>	<u>Express Forcer</u>	<u>1</u>
	<u>present</u>	<u>présente</u>	<u>vorhanden</u>	<u>presente</u>	<u>Fonda</u>	<u>9</u>
26.	VG	<u>Resistance to</u>	<u>Résistance à</u>	<u>Resistenz gegen</u>	<u>Resistencia a</u>	
(+)	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>		
	<u>- Race Pb: 1</u>	<u>- Pathotype Pb: 1</u>	<u>- Pathotyp Pb: 1</u>	<u>- Raza Pb: 1</u>		
QL	<u>absent</u>	<u>absente</u>	<u>fehlend</u>	<u>ausente</u>	<u>Express Forcer</u>	<u>1</u>
	<u>present</u>	<u>présente</u>	<u>vorhanden</u>	<u>presente</u>	<u>Fonda</u>	<u>9</u>
27.	VG	<u>Resistance to</u>	<u>Résistance à</u>	<u>Resistenz gegen</u>	<u>Resistencia a</u>	
(+)	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>		
	<u>- Race Pb: 2</u>	<u>- Pathotype Pb: 2</u>	<u>- Pathotyp Pb: 2</u>	<u>- Raza Pb: 2</u>		
QL	<u>absent</u>	<u>absente</u>	<u>fehlend</u>	<u>ausente</u>	<u>Express Forcer, Fonda</u>	<u>1</u>
	<u>present</u>	<u>présente</u>	<u>vorhanden</u>	<u>presente</u>		<u>9</u>
28.	VG	<u>Resistance to</u>	<u>Résistance à</u>	<u>Resistenz gegen</u>	<u>Resistencia a</u>	
(+)	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>	<u><i>Plasmodiophora brassicae</i> (Pb)</u>		
	<u>- Race Pb: 3</u>	<u>- Pathotype Pb: 3</u>	<u>- Pathotyp Pb: 3</u>	<u>- Raza Pb: 3</u>		
QL	<u>absent</u>	<u>absente</u>	<u>fehlend</u>	<u>ausente</u>	<u>Express Forcer</u>	<u>1</u>
	<u>present</u>	<u>présente</u>	<u>vorhanden</u>	<u>presente</u>	<u>Fonda</u>	<u>9</u>

Proposed addition of new explanation Ad. 25 to 28 "Resistance to *Plasmodiophora brassicae* (Pb)" Races Pb: 0, 1, 2 and 3

Ad. 25 to 28: Resistance to *Plasmodiophora brassicae* (Pb) Races Pb: 0, 1, 2 and 3

1.	Pathogen	<i>Plasmodiophora brassicae</i>
2.	Quarantine status	no
3.	Host species	<i>Brassica oleracea</i>
4.	Source of inoculum	Naktuinbouw ³ (NL)
5.	Isolate	Race Pb: 0, Pb: 1, Pb: 2 and Pb: 3
6.	Establishment isolate identity	with genetically defined differentials from Naktuinbouw (NL)
7.	Establishment pathogenicity	symptoms on susceptible <i>Brassica oleracea</i> varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	Plant roots
8.2	Multiplication variety	Bartolo (WC), Granaat (CC) ²
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 inoculum	Microscopic count
8.8	Shelf life/viability inoculum	Frozen 3 years, room temp 1-2 days
9.	Format of the test	
9.1	Number of plants per genotype	20 plants per genotype
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 Bejo 051632 (WC)
9.5	Test facility	glasshouse
9.6	Temperature	20-22°C
9.7	Light	Natural, extended to 16 h if needed
9.9	Special measures	saturated soil in the first week, and keep the soil wet to decrease the soil temperature, but keep in mind that a moderate amount of water is required to prevent rotting,
10.	Inoculation	
10.1	Preparation inoculum	Symptomatic roots are homogenized ca. 1 min in a blender. Dilute clubs 1:4 with demineralised water. Prevent overheating of the suspension by blending longer than 1 minute. (Beware: longer periods of blending 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	Pipetting of 2 ml to the base of each seedling
10.5	First observation	4 weeks after inoculation (visual)
10.6	Second observation	5 weeks after inoculation (visual)
10.7	Final observations	6 weeks after inoculation (visual)
11.	Observations	

³ Naktuinbouw: resistentie@naktuinbouw.nl

² WC=White cabbage, CC=Chinese cabbage, RC=Red cabbage, CF=Cauliflower

11.1	Method	Visual: observation of severe galling and growth retardation Destructive: observation on a 0-3 scale for galling
11.2	Obervation scale	grade 0 = no swellings or a few small spheroid galls grade 1 = very slight swelling, usually confined to the lateral roots grade 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 grade 3 = severe swelling on lateral and/or tap roots
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12.	Interpretation of data in terms of UPOV characteristic states	absent [1] symptoms grade 2 and 3. present [9] symptoms grade 0 and 1
13.	Critical control points	



0 = no galling



1 = a few small galls



2 = moderate galling



3 = severe galling



2 = slight swelling of the main root, no lateral roots

[End of document]