UPOV
International Union for the Protection of New Varieties of Plants

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Technical Working Party for Vegetables

TWV/59/16

Fifty-Ninth Session
Virtual meeting, May 5 to 8, 2025

Original: English
Date: April 11, 2025

PARTIAL REVISION OF THE TEST GUIDELINES FOR CAULIFLOWER

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 Cauliflower (document TG/45/7 Rev. 2).

2. The Technical Working Party for Vegetables (TWV), at its fifty-eighth session[1], agreed that the Test Guidelines for Cauliflower (*Brassica oleracea* L. convar *botrytis* (L.) Alef.var. *botrytis* L.*)* be partially revised (see document TWV/58/11 "Report", Annex II).

3. The following changes are proposed:

(a) Revision of characteristic 28 "Male sterility"
(b) Revision of explanation Ad. 28 "Male sterility"
(c) Revision of the Technical Questionnaire, Section TQ 4. "Information on the breeding scheme and propagation of the variety"
(d) Addition of Characteristic 29 "Resistance to *Plasmodiophora brassicae* (Pb)", including example varieties
(e) Addition of explanation Ad. 29 "Resistance to *Plasmodiophora brassicae* (Pb)"

4. The proposed changes are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

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

Proposed revision of characteristic 28 “Male sterility”

Current wording

28. (*) (+)	MS/VS	Male sterility	Stérilité mâle	Männliche Sterilität	Androesterilidad		
QN		absent	absente	fehlend	ausente	Alpha 2, Flora Blanca	1
		partial	partielle	partiell	parcial	Dunvez, Odegwen	2
		total	totale	vollständig	total	Aviron, Bodilis	3

Proposed new wording

28. (*) (+)	MS/VS	Male sterility	Stérilité mâle	Männliche Sterilität	Androesterilidad		
QN		absent	absente	fehlend	ausente	Alpha 2, Flora Blanca	1
		partially present	partielle	partiell	parcial	Dunvez, Odegwen	2
		totally present	totale	vollständig	total	Aviron, Bodilis	3

Proposed revision of explanation Ad. 28 “Male sterility”

Current wording

Ad. 28: Male sterility

To be tested in a field trial and/or in a DNA marker test[2].

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

Field trial:

Observations should be made on fully opened flowers. Tapping or shaking the flowering stem will release pollen, which, if present, can be observed on dark colored paper or card. The absence of pollen production is an indication of male sterility. The presence of pollen production is an indication of male fertility.

Absent: >70% of the plants fertile (open-pollinated varieties or hybrid varieties produced with self-incompatibility system)
Partial: 30% to 70% of the plants fertile (hybrid varieties produced with genic male sterility, in heterozygous state)
Total: < 30% of the plants fertile (hybrid varieties produced with cytoplasmic male sterility)

[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.

male fertile (pollen present)

male sterile (pollen absent)

DNA marker test and/or field trial:

Varieties declared male fertile (state 1) or total male sterile (state 3) in the TQ, can be examined in a field trial or in a DNA marker test.
Varieties with partial male sterility (state 2) and vegetatively propagated, total male sterile lines (state 3) cannot be examined in a DNA marker test but must be observed in a field trial.

It should be noted that lines exist which are male sterile due to the homozygous recessive monogenic male sterility (GMS) gene. These lines are used for the production of hybrids which then will be male fertile. However when a heterozygous mother line is used, the produced hybrids will be partially male sterile (state 2). Due to their nature these lines have to be propagated vegetatively. They are male sterile but do not have the DNA marker for the presence of cytoplasmic male sterility (CMS). So vegetatively propagated male sterile lines cannot be examined in a DNA marker test but must be observed in a field trial.

In cases where only a DNA marker test is allowed (state 1 and state 3 seed-propagated varieties) and the CMS maker appears to be absent, the variety is expected to have male fertile flowers. In cases where the CMS marker is present, the variety is expected to have male sterile flowers. All varieties declared partially sterile (state 2) and vegetatively propagated lines declared total male sterile (state 3) should be tested in a field trial.

In cases where 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 or is segregating due to another mechanism.

The marker test can be performed in multiplex with the marker test for flower color (Ad. 25).

Proposed new wording

Ad. 28: Male sterility

To be tested in a field trial and/or in a DNA marker test[3].

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

Field trial:

Observations should be made on fully opened flowers. Tapping or shaking the flowering stem will release pollen, which, if present, can be observed on dark colored paper or card. The absence of pollen production is an indication of male sterility. The presence of pollen production is an indication of male fertility.

[3] 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.

Absent: ~~>70% of the~~ all plants with male fertile flowers ~~(open-pollinated varieties or hybrid varieties produced with self-incompatibility system)~~

Partially present: ~~30% to 70%~~ 50% of the plants with male fertile flowers and 50% plants with male sterile flowers ~~(hybrid varieties produced with genic male sterility, in heterozygous state~~

Totally present: ~~< 30% of the~~ all plants ~~fertile~~ with male sterile flowers ~~(hybrid varieties produced with cytoplasmic male sterility)~~

Hybrids produced with a motherline which is heterozygous for genic male sterility (GMS), may segregate for male sterility. Due to their nature such motherlines have to be propagated vegetatively.
If the segregation occurs in the predicted manner, the hybrid should be classified as partially present (state 2).

male fertile (pollen present)

male sterile (pollen absent)

DNA marker test and/or field trial:

Varieties declared male fertile (state 1) or total male sterile (state 3) in the TQ, can be examined in a field trial or in a DNA marker test.
Varieties with partial male sterility (state 2) and vegetatively propagated, total male sterile lines (state 3) cannot be examined in a DNA marker test but must be observed in a field trial.

It should be noted that lines exist which are male sterile due to the homozygous recessive monogenic male sterility (GMS) gene. These lines are used for the production of hybrids which then will be male fertile. However when a heterozygous mother line is used, the produced hybrids will be partially male sterile (state 2). Due to their nature these lines have to be propagated vegetatively. They are male sterile but do not have the DNA marker for the presence of cytoplasmic male sterility (CMS). So vegetatively propagated male sterile lines cannot be examined in a DNA marker test but must be observed in a field trial.

In cases where only a DNA marker test is allowed (state 1 and state 3 seed-propagated varieties) and the CMS maker appears to be absent, the variety is expected to have male fertile flowers. In cases where the CMS marker is present, the variety is expected to have male sterile flowers. All varieties declared partially sterile (state 2) and vegetatively propagated lines declared total male sterile (state 3) should be tested in a field trial.

In cases where 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 or is segregating due to another mechanism.

The marker test can be performed in multiplex with the marker test for flower color (Ad. 25).

Proposed revision of the Technical Questionnaire, Section TQ 4. “Information on the breeding scheme and propagation of the variety”

Current wording

4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]
(please state parent varieties)
(b) partially known cross [ ]
(please state known parent variety(ies))
(c) unknown cross [ ]

4.1.2 Mutation [ ]
(please state parent variety)

4.1.3 Discovery and development [ ]
(please state where and when discovered and how developed)

4.1.4 Other [ ]
(please provide details)

4.2 Method of propagating the variety

4.2.1 Seed-propagated varieties

(a) Self-pollination [ ]
(b) Cross-pollination
(i) population [ ]
(ii) synthetic variety [ ]
(c) Hybrid [ ]
(d) Other [ ]
(please provide details)

4.2.2 Other [ ]
(please provide details)

Proposed new wording

5. The Technical Questionnaire needs to be revised and should contain the following items:

Type of material:

a) hybrid [ ]
(i) seed propagated parents [ ]
(ii) one of more vegetatively propagated parent [ ]
b) cross-pollinated variety [ ]
c) parental line [ ]

Method of propagation of the variety

a) seed propagated [ ]
b) vegetatively propagated [ ]

Proposed addition of new Characteristic 29 “Resistance to *Plasmodiophora brassicae* (Pb)”, including example varieties at the end of Table of Characteristics

29 **(+)**	**VS**	**Resistance to *Plasmodiophora brassicae* (Pb)**	**Résistance à *Plasmodiophora brassicae* (Pb)**	**Resistenz gegen *Plasmodiophora brassicae* (Pb)**	**Resistencia a *Plasmodiophora brassicae* (Pb)**		
29.1	**VS**	**– Race Pb: 0**	**– Pathotype Pb: 0**	**– Pathotyp Pb: 0**	**– Raza Pb: 0**		
QL		absent	absente	fehlend	ausente	Freedom	1
		present	présente	vorhanden	presente	Clapton	9
29.2	**VS**	**– Race Pb: 1**	**– Pathotype Pb: 1**	**– Pathotyp Pb: 1**	**– Raza Pb: 1**		
QL		absent	absente	fehlend	ausente	Freedom	1
		present	présente	vorhanden	presente	Clapton	9
29.3	**VS**	**– Race Pb: 2**	**– Pathotype Pb: 2**	**– Pathotyp Pb: 2**	**– Raza Pb: 2**		
QL		absent	absente	fehlend	ausente	Freedom, Clapton	1
		present	présente	vorhanden	presente		9
29.4	**VS**	**– Race Pb: 3**	**– Pathotype Pb: 3**	**– Pathotyp Pb: 3**	**– Raza Pb: 3**		
QL		absent	absente	fehlend	ausente	Freedom	1
		present	présente	vorhanden	presente	Clapton	9

Proposed addition of an explanation Ad. 29 "Resistance to *Plasmodiophora brassicae* (Pb)" in Chapter 8.2 "Explanations for individual characteristics"

Ad. 29: Resistance to *Plasmodiophora brassicae* (Pb)

1.	Pathogen	*Plasmodiophora brassicae*
2.	Quarantine status	no
3.	Host species	*Brassica oleracea*
4.	Source of inoculum	Naktuinbouw[4] (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 inoculum	Microscopic count
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.

[4] Naktuinbouw: resistentie@naktuinbouw.nl

[3] WC=White cabbage, CC=Chinese cabbage, RC=Red cabbage, CF=Cauliflower

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.


0 = no galling


1 = a few small galls


2 = moderate galling


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


3 = severe galling

[End of document]