UPOV
International Union for the Protection of New Varieties of Plants

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PARTIAL REVISION OF THE TEST GUIDELINES FOR CAULIFLOWER

Document prepared by an expert from the Netherlands

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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. The Technical Working Party for Vegetables (TWV), at its fifty-sixth 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/56/22 "Report", Annex II).

3. The following changes are proposed:

(a) Revision of Characteristic 25 "Flower: color"
(b) Addition of new explanation Ad. 25 Characteristic 25 "Flower: color"
(c) Revision of explanation Ad. 28 "Male sterility"
(d) Addition of new Characteristics 29 to 32 "Resistance to *Plasmodiophora brassicae* (Pb)" Races Pb: 0, 1, 2 and 3 (clubroot)
(e) Addition of new explanation Ad. 29 to 32 "Resistance to *Plasmodiophora brassicae* (Pb)" Races Pb: 0, 1, 2 and 3
(f) Addition of references to Chapter 9. "Literature"

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 25 “Flower: color”

Current wording

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
25. (*)	**VG**	**Flower: color**	**Fleur : couleur**	**Blüte: Farbe**	**Flor: color**		
QL		white	blanche	weiß	blanco	Bruce, Ecrin	1
		yellow	jaune	gelb	amarillo	Flora Blanca, Lecerf	2

Proposed new wording

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
25. (*) (+)	**~~VG~~ VS/ MS**	**Flower: color**	**Fleur : couleur**	**Blüte: Farbe**	**Flor: color**		
QL		white	blanche	weiß	blanco	Bruce, Ecrin	1
		yellow	jaune	gelb	amarillo	Flora Blanca, Lecerf	2

Proposed addition of new explanation Ad. 25 Characteristic 25 “Flower: color”

Ad 25: Flower: color

DNA marker test for examination of the flower color.

DNA marker test:

The gene CCD4 is responsible for the white petal color in *Brassica oleracea* L. convar *botrytis* (L.) Alef. var. *botrytis* L. Functional loss of this gene is responsible for the yellow petal color. The markers corresponding with the functional gene and nonfunctional gene are based on 3 SNP’s on position ~1296bp in the genes (Han et al. 2019).
The markers can be performed in multiplex with the marker for male sterility (Ad. 28).
The presence of the functional or nonfunctional CCD4 gene can be detected by the described co-dominant markers.

Specific aspects:

1.	Characteristic	Flower: color
2.	Functional gene	Functional CCD4 gene : white Nonfunctional CCD4 gene: yellow
3.1	Primers	Tm of the primers is ~57°C Forward Primer: “5-CTGGATTCAACATCATTCACG CT-3’ Reverse Primer: ‘5-CGGTGACGAGATCGATCTTCA-3’
3.2	Probes	White Probe: ‘5-Fluorophore-ATCGCTCCAAATATTATGT-Quencer-3’ Yellow Probe: ‘5-Fluorophore-GCTCCGAACGTTATGT-Quencer-3’
		The probes are MGB probes (Applied biosystems) or XS probes (Biolegio). The Tm of the probes must be ordered at 67°C. Fluorophores can be modified according to compatibility with the filters on the real-time PCR machine.
4.	Format of the test	
4.1	Number of plants per genotype	at least 20 plants
4.2	Control varieties	Homozygous allele for functional CCD4 gene (white petal color) present: Ecrin Heterozygous functional and nonfunctional CCD4 gene present (variety is white): Bruce Homozygous allele for nonfunctional CCD4 gene (yellow petal color) present: Magnifico
6.	PCR conditions (mastermix dependent)	1. Initial denaturation step 10 min 95 °C 2. 40 cycles 15 sec 95 °C and 1 min 60°C. Every cycle ends with a plate reading.
8.	Interpretation of test results	
	White (1):	Probe for functional CCD4 gene (white petal color) is homozygous present, variety has white flowers. Both probes are present (heterozygous), the variety has white flower.
	Yellow (2)	Probe for nonfunctional CCD4 gene (yellow petal color) is homozygous present, the variety has yellow flowers. 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 white or yellow flowers due to another mechanism.

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

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.

Field trial:

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 heterozygotic state)
Total	=	< 30% of the plants fertile (hybrid varieties produced with cytoplasmic male sterility)

DNA marker test and/or field trial:

All varieties declared total male sterile (state 3) in the TQ can be examined in a field trial or in a DNA marker test[1]. 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), partial sterile or fertile. All varieties declared fertile or partial male sterile are to be tested in a field trial.

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

Proposed new wording

Ad. 28: Male sterility

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

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

Field trial:

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~~tic~~ state)
Total:	< 30% of the plants fertile (hybrid varieties produced with cytoplasmic male sterility)

DNA marker test and/or field trial:

~~All~~ 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[2].
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 ~~normally~~ 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 CMS male sterility. So vegetatively propagated male sterile lines cannot be examined in a DNA marker test but must be observed in a field trial.
For the cases where only a DNA marker test is allowed (state 1 and state 3 seed propagated varieties), in the case of a DNA marker test, if the CMS marker appears to be not present, ~~a field trial should be perfomed 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 is present, the variety is expected to have male sterile flowers. ~~All varieties declared fertile are to be tested in a field trial.~~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 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 or is segregating due to another mechanism.

In case of a field trial, the type of observation is VS. In 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 29 to 32 “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
29. **(+)**	**VG**	**Resistance to *Plasmodiophora brassicae* (Pb) – Race Pb: 0**	**Résistance à *Plasmodiophora brassicae* (Pb) – Pathotype Pb: 0**	**Resistenz gegen *Plasmodiophora brassicae* (Pb) – Pathotyp Pb: 0**	**Resistencia a *Plasmodiophora brassicae* (Pb) – Raza Pb: 0**		
QL		absent	absente	fehlend	ausente	Fremont	1
		present	présente	vorhanden	presente	Clapton	9
30. **(+)**	**VG**	**Resistance to *Plasmodiophora brassicae* (Pb) – Race Pb: 1**	**Résistance à *Plasmodiophora brassicae* (Pb) – Pathotype Pb: 1**	**Resistenz gegen *Plasmodiophora brassicae* (Pb) – Pathotyp Pb: 1**	**Resistencia a *Plasmodiophora brassicae* (Pb) – Raza Pb: 1**		
QL		absent	absente	fehlend	ausente	Fremont	1
		present	présente	vorhanden	presente	Clapton	9
31. **(+)**	**VG**	**Resistance to *Plasmodiophora brassicae* (Pb) – Race Pb: 2**	**Résistance à *Plasmodiophora brassicae* (Pb) – Pathotype Pb: 2**	**Resistenz gegen *Plasmodiophora brassicae* (Pb) – Pathotyp Pb: 2**	**Resistencia a *Plasmodiophora brassicae* (Pb) – Raza Pb: 2**		
QL		absent	absente	fehlend	ausente	Fremont, Clapton	1
		present	présente	vorhanden	presente		9
32. **(+)**	**VG**	**Resistance to *Plasmodiophora brassicae* (Pb) – Race Pb: 3**	**Résistance à *Plasmodiophora brassicae* (Pb) – Pathotype Pb: 3**	**Resistenz gegen *Plasmodiophora brassicae* (Pb) – Pathotyp Pb: 3**	**Resistencia a *Plasmodiophora brassicae* (Pb) – Raza Pb: 3**		
QL		absent	absente	fehlend	ausente	Fremont	1
		present	présente	vorhanden	presente	Clapton	9

Proposed addition of new explanation Ad. 29 to 32 “Resistance to Plasmodiophora brassicae (Pb)” Races Pb: 0, 1, 2 and 3

Ad. 29 to 32: Resistance to *Plasmodiophora brassicae* (Pb) Races Pb: 0, 1, 2 and 3

1.	Pathogen	*Plasmodiophora brassicae*
2.	Quarantine status	no
3.	Host species	*Brassica oleracea*
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 *Brassica oleracea* 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)

[3] Naktuinbouw: resistentie@naktuinbouw.nl

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

11.	Observations	
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

Proposed addition of references to Chapter 9. “Literature”

Current wording

9. LITERATURE

Fujime, Y., 1983: Studies on Thermal Conditions of Curd Formation and Development in Cauliflower and Broccoli, with Special Reference to Abnormal Curd Development. Memoires of Faculty of Agriculture, Kagawa University, No. 40, February 1983, pp. 1-123, JP.

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Wiebe, H.J., 1975: The Morphological development of cauliflower and broccoli cultivars depending on temperature. Sci. Hort. 3, pp. 95-101.

Wiebe, H.J., 1981: Influence of transplant characteristics and growing conditions on curd size (buttoning) of cauliflower. Acta Hort. 122, pp. 99-105.

Proposed new wording

9. LITERATURE

Fengqing Han, Huilin Cui, Bin Zhang, Xiaoping Liu, Limei Yang, Mu Zhuang, Honghao Lv, Zhansheng Li, Yong Wang, Zhiyuan Fang, Jianghua Song and Yangyong Zhang. Map-based cloning and characterization of BoCCD4, a gene responsible for white/yellow petal color in B. oleracea BMC Genomics (2019) 20:242

Fujime, Y., 1983: Studies on Thermal Conditions of Curd Formation and Development in Cauliflower and Broccoli, with Special Reference to Abnormal Curd Development. Memoires of Faculty of Agriculture, Kagawa University, No. 40, February 1983, pp. 1-123, JP.

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