UPOV
International Union for the Protection of New Varieties of Plants

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Technical Committee **TC/59/13**

Fifty-Ninth Session
Geneva, October 23 and 24, 2023

Original: English
Date: September 12, 2023

PARTIAL REVISION OF THE TEST GUIDELINES FOR CAULIFLOWER

Document prepared by 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-seventh session[1], considered a proposal for a partial revision of the Test Guidelines for Cauliflower (*Brassica oleracea* L. convar *botrytis* (L.) Alef. var. *botrytis* L.) on the basis of documents TG/45/7 Rev. and TWV/57/20 "*Partial revision of the Test Guidelines for Cauliflower*" and proposed the following changes (see document TWV/57/26 "*Report*", paragraph 63):

(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 references to Chapter 9. "Literature"

3. The proposed new wording is presented below. The proposed changes are presented in highlight and underline (insertion) and ~~strikethrough~~ (deletion) in the Annex to this document (in English only).

Proposed revision of Characteristic 25 "Flower: color"

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
25. (*) (+)	**VG/ 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

[1] held in Antalya, Türkiye, from May 1 to 5, 2023

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

Ad 25: Flower: color

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

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

Field trial:

Check de color of flowers.

1	2
white	yellow

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.

Proposed revision of explanation Ad. 28 “Male sterility”

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:

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)

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 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), if the CMS marker appears to be not present, 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 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.

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

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[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 references to Chapter 9. “Literature”

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, 2019: Map-based cloning and characterization of BoCCD4, a gene responsible for white/yellow petal color in *B. oleracea* BMC Genomics. 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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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.

[Annex follows]

PROPOSED CHANGES PRESENTED IN HIGHLIGHT
(in English only)

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	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”

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

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male fertile (pollen present)

male sterile (pollen absent)

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.
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), 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 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.

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[End of Annex and of document]