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| International Union for the Protection of New Varieties of Plants |  |

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| Technical Working Party for VegetablesFifty-Seventh SessionAntalya, Türkiye, May 1 to 5, 2023 | TWV/57/20Original: EnglishDate: March 28, 2023 |

Partial revision of the Test Guidelines for cauliflower

Document prepared by an expert from the Netherlands

Disclaimer: this document does not represent UPOV policies or guidance

 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.).

 The Technical Working Party for Vegetables (TWV), at its fifty-sixth session[[1]](#footnote-2), 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).

 The following changes are proposed:

1. Revision of Characteristic 25 “Flower: color”
2. Addition of new explanation Ad. 25 Characteristic 25 “Flower: color”
3. Revision of explanation Ad. 28 “Male sterility”
4. Addition of new Characteristics 29 to 32 “Resistance to *Plasmodiophora brassicae* (Pb)” Races Pb: 0, 1, 2 and 3 (clubroot)
5. Addition of new explanation Ad. 29 to 32 “Resistance to *Plasmodiophora brassicae* (Pb)” Races Pb: 0, 1, 2 and 3
6. Addition of references to Chapter 9. “Literature”

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

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 : whiteNonfunctional CCD4 gene: yellow  |
| 3.1 | Primers | Tm of the primers is ~57°CForward 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: EcrinHeterozygous functional and nonfunctional CCD4 gene present (variety is white): BruceHomozygous allele for nonfunctional CCD4 gene (yellow petal color) present: Magnifico |
| 6. | PCR conditions(mastermix dependent) | 1. Initial denaturation step 10 min 95 °C2. 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 test1. 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]](#footnote-3).

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.

Proposed addition of new Characteristics 29 to 32 “Resistance to *Plasmodiophora brassicae* (Pb)” Races Pb: 0, 1, 2 and 3 (clubroot)

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| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 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]](#footnote-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) |
| 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 (107 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) 2Resistant 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 107 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 |  |
| 11.1 | Method | Visual: observation of severe galling and growth retardationDestructive: 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 rootsgrade 2 = moderate swelling on lateral and/or tap roots orslight 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 |  |





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

Proposed addition of references to Chapter 9. “Literature”

*Current wording*

# 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.

Gray, A.R., 1989: Taxonomy and Evolution of Broccoli and Cauliflower. Baileya 23 (1), pp. 28-46.

Nieuwhof, M., 1969: Cole Crops. World Crops Books: Leonard Hill, London, GB.

Sadik, S., 1962: Morphology of the curd of cauliflower. Amer. Bot. 49, pp. 290-297.

Tsunoda, S., Hinata, K., and Gomez-Campo, C., 1980: Brassica Crops and Wild Allies. Biology and Breeding, Japan Scientific Societies Press, Tokyo, JP.

Wiebe, H.J., 1972/73: Wirkung von Temperatur und Licht auf Wachstum und Entwicklung von Blumenkohl. Gartenbauwissenschaft 37, pp. 165-178, 37, pp. 293-303, 37, pp. 455-469, 38, pp. 263-279, 38, pp. 433-440.

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*

# 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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1. organized by electronic means, from April 18 to 22, 2022 [↑](#footnote-ref-2)
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. [↑](#footnote-ref-3)
3. Naktuinbouw: resistentie@naktuinbouw.nl

2 WC=White cabbage, CC=Chinese cabbage, RC=Red cabbage, CF=Cauliflower [↑](#footnote-ref-4)