CPVO · OCVV
Community Plant Variety Office
Office Communautaire des Variétés Végétales

PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Brassica oleracea L. convar. *botrytis* (L.) Alef. var. *botrytis* L.

CAULIFLOWER

UPOV Code: BRASS_OLE_GBB

Adopted on 11/04/2024

Entry into force on 15/01/2024

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CPVO-TP/045/2 Rev.3

1. SUBJECT OF THE PROTOCOL AND REPORTING

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of ***Brassica oleracea*** **L. convar.** ***botrytis*** **(L.) Alef. var.** ***botrytis*** **L..**

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on documents agreed by the International Union for the Protection of New Varieties of Plants (UPOV), such as the General Introduction to DUS (UPOV Document TG/1/3 http://www.upov.int/export/sites/upov/resource/en/tg_1_3.pdf), its associated TGP documents (http://www.upov.int/tgp/en/) and the relevant UPOV Test Guideline TG/45/7 Rev. 2 dated 24/10/2023 (https://www.upov.int/edocs/tgdocs/en/tg045.pdf) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **15.01.2024**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

In cases where the Office requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process to be carried out at the moment of this request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

1.3 Reporting between Examination Office and CPVO and Liaison with Applicant

1.3.1 Reporting between Examination Office and CPVO

The Examination Office shall deliver to the CPVO a preliminary report ("the preliminary report") no later than two weeks after the date of the request for technical examination by the CPVO.

The Examination Office shall also deliver to the CPVO a report relating to each growing period ("the interim report") and, when the Examination Office considers the results of the technical examination to be adequate to evaluate the variety or the CPVO so requests, a report relating to the examination ("the final report").

The final report shall state the opinion of the Examination Office on the distinctness, uniformity and stability of the variety. Where it considers those criteria to be satisfied, or where the CPVO so requests, a description of the variety shall be added to the report. If a report is negative the Examination Office shall set out the detailed reasons for its findings.

The interim and the final reports shall be delivered to the CPVO as soon as possible and no later than on the deadlines as laid down in the designation agreement.

1.3.2 Informing on problems in the DUS test

If problems arise during the course of the test the CPVO should be informed immediately so that the information can be passed on to the applicant. Subject to prior permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

1.3.3 Sample keeping in case of problems

If the technical examination has resulted in a negative report, the CPVO shall inform the Examination Office as soon as possible in case that a representative sample of any relevant testing material shall be kept.

2. MATERIAL REQUIRED

2.1 Plant material requirements

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on https://public.plantvarieties.eu/publication in the special issue S2 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

2.2 Informing the applicant of plant material requirements

The CPVO informs the applicant that

- he is responsible for ensuring compliance with any customs and plant health requirements.
- the plant material supplied should be visibly healthy, not lacking in vigour, nor affected by any important pest or disease.
- the plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

2.3 Informing about problems on the submission of material

The Examination Office shall report to the CPVO immediately in cases where the test material of the candidate variety has not arrived in time or in cases where the material submitted does not fulfil the conditions laid down in the request for material issued by the CPVO.

In cases where the examination office encounters difficulties to obtain plant material of reference varieties the CPVO should be informed.

3. METHOD OF EXAMINATION

3.1 Number of growing cycles

The minimum duration of tests should normally be two growing cycles.

The two independent growing cycles should be in the form of two separate plantings.

3.2 Testing Place

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness" http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf.

3.3 Conditions for Conducting the Examination

The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

Stage of development for the assessment
The optimum stage of development for the assessment of each characteristic is indicated by a number in the third column of the Table of Characteristics. The stages of development denoted by each number are described in Chapter 8.3

3.4 Test design

3.4.1 Each test should be designed to result in a total of at least 60 plants, which should be divided between at least 2 replicates.

3.4.2 The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.5 Additional tests

In accordance with Article 83(3) of Council Regulation No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, an additional test may be undertaken providing that a technically acceptable test procedure can be devised.

Additional tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

3.6 Constitution and maintenance of a variety collection

The process for the constitution and the maintenance of a variety collection can be summarized as follows:

Step 1: Making an inventory of the varieties of common knowledge

Step 2: Establishing a collection ("variety collection") of varieties of common knowledge which are relevant for the examination of distinctness of candidate varieties

Step 3: Selecting the varieties from the variety collection which need to be included in the growing trial or other tests for the examination of distinctness of a particular candidate variety.

3.6.1 Forms of variety collection
The variety collection shall comprise variety descriptions and living plant material, thus a living reference collection. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database.

3.6.2 Living Plant Material
The EO shall collect and maintain living plant material of varieties of the species concerned in the variety collection.

3.6.3 Range of the variety collection
The living variety collection shall cover at least those varieties that are suitable to climatic conditions of a respective EO.

3.6.4 Making an inventory of varieties of common knowledge for inclusion in the variety collection
The inventory shall take into account the list of protected varieties and the official, or other, registers of varieties, in particular:

The inventory shall include varieties protected under National PBR (UPOV contracting parties) and Community PBR, varieties registered in the Common Catalogue, the OECD list, the Conservation variety list and varieties in trade or in commercial registers for those species not covered by a National or the Common Catalogue.

3.6.5 Maintenance and renewal/update of a living variety collection
The EO shall maintain seeds in conditions which will ensure germination and viability, periodical checks, and renewal as required. For the renewal of existing living material the identity of replacement living plant material shall be verified by conducting side-by-side plot comparisons between the material in the collection and the new material.

4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

The prescribed procedure is to assess distinctness, uniformity and stability in a growing trial.

4.1 Distinctness

4.1.1 General recommendations
It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 9 'Examining Distinctness' (http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

Further guidance is provided in documents TGP/9 "Examining Distinctness" and TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability".

4.1.2 Consistent differences
The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Technical Protocols are familiar with the recommendations contained in the UPOV-General Introduction to DUS prior to making decisions regarding distinctness.

4.1.4 Number of plants/parts of plants to be examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 20 plants or parts taken from each of 20 plants and any other observations made on all plants in the test, disregarding any off-type plants. In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 20

4.1.5 Method of observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the third column of the Table of Characteristics (see document TGP/9 "Examining Distinctness", Section 4 "Observation of characteristics"):

MG: single measurement of a group of plants or parts of plants
MS: measurement of a number of individual plants or parts of plants
VG: visual assessment by a single observation of a group of plants or parts of plants
VS: visual assessment by observation of individual plants or parts of plants

Type of observation: visual (V) or measurement (M)
"Visual" observation (V) is an observation made on the basis of the expert's judgment. For the purposes of this document, "visual" observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. colour charts). Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

Type of record: for a group of plants (G) or for single, individual plants (S)
For the purposes of distinctness, observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded as records for a number of single, individual plants or parts of plants (S). In most cases, "G" provides a single record per variety and it is not possible or necessary to apply statistical methods in a plant-by-plant analysis for the assessment of distinctness."

In cases where more than one method of observing the characteristic is indicated in the Table of Characteristics (e.g. VG/MG), guidance on selecting an appropriate method is provided in document TGP/9, Section 4.2.

4.2 Uniformity

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 10 'Examining Uniformity' (http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

The assessment of uniformity should be according to the recommendations for cross-pollinated varieties in the UPOV-General Introduction to DUS.

For the assessment of uniformity of single cross hybrids and inbred lines, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 60 plants, 2 off-types are allowed.

In addition, a population standard of 2% and an acceptance probability of at least 95% should be applied for **aberrant plants**. The definition of aberrant plants is explained in Chapter 8.3. In the case of a sample size of 60 plants, 3 aberrant plants are allowed.

In addition, for single cross hybrids, a population standard of 3% and an acceptance probability of at least 95% should be applied for inbred plants obviously resulting from the selfing of a parent line. In the case of a sample size of 60 plants, 4 inbred plants are allowed.

4.3 Stability

4.3.1 It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 11 'Examining Stability' (http://www.upov.int/edocs/tgpdocs/en/tgp_11.pdf).

In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

5. GROUPING OF VARIETIES AND ORGANIZATION OF THE GROWING TRIAL

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

a) Seedling: anthocyanin coloration of hypocotyl (characteristic 1)

b) Curd: colour (characteristic 21)

c) Flower: colour (characteristic 25)

d) Earliness in spring planting (characteristic 26)

e) Earliness in summer planting (characteristic 27)

f) Male sterility (characteristic 28)

5.4 If other characteristics than those from the TP are used for the selection of varieties to be included into the growing trial, the EO shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.

6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

6.1 Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted or by specific legislation on plant health. In the latter case, the CPVO should be informed.
The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N°874/2009, to insert additional characteristics and their expressions in respect of a variety.

6.1.2 **Technical Protocols with asterisked characteristics (only for certain vegetable species)**
In the case of disease resistance characteristics, only those resistances marked with an asterisk (*) in the CPVO column are compulsory.

States of expression and corresponding notes
In the case of qualitative and pseudo-qualitative characteristics, all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines may be abbreviated as follows:

State	Note
small	3
medium	5
large	7

However, it should be noted that all of the following 9 states of expression exist to describe varieties and should be used as appropriate:

State	Note
very small	1
very small to small	2
small	3
small to medium	4
medium	5
medium to large	6
large	7
large to very large	8
very large	9

6.2 Example Varieties

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

6.3 Legend

For the CPVO N° column:

G	Grouping characteristic	– see Chapter 5
(*)	Asterisked characteristic	– see Chapter 6.1.2 (only for certain vegetable species)
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	See Explanations on the Table of Characteristics in Chapter 8.2	

For the UPOV N° column:
The numbering of the characteristics is provided as a reference to the ad hoc UPOV guideline.

(*)	UPOV Asterisked characteristic	– Characteristics that are important for the international harmonisation of variety descriptions.

For column "stage, method":

MG, MS, VG, VS		– see Chapter 4.1.5
(a)-(b)	See Explanations on the Table of Characteristics in Chapter 8.1	

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1.	**1. (*)**	**VG**	**Seedling: anthocyanin coloration of hypocotyl**		
QL			absent	Brio	1
G			present	Ciren, Dominant	9
2.	**2.**	**VG/MG**	**Plant: height (at time of harvest)**		
QN		**(a)**	very short		1
			short	Luxor, Opaal	3
			medium	Fastman, Mexico	5
			tall	Neven, Sirente	7
			very tall	Calisa, Paradiso	9
3.	**3.**	**VG/MG**	**Stem: length (up to insertion of first leaf)**		
QN		**(a)**	short	Mexico, Opaal	3
			medium	Nautilus	5
			long	Neven, Paradiso	7
4.	**4.**	**VG**	**Leaf: attitude**		
(+)	**(*)**	**(a)**	erect	Igloo, Paradiso	1
QN			semi-erect	Erfurter Zwerg, Fastman	3
			horizontal	Isabel, Opaal	5
5.	**5. (*)**	**VG/MS**	**Leaf: length**		
QN		**(a)**	very short		1
			short	Nagano, Opaal	3
			medium	Aviso	5
			long	Géant de Naples tardif, Snow March, Memphis	7
			very long	Magnifico, Paradiso	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6.	**6.**	**VG/MS**	**Leaf: width**		
QN	**(*)**	**(a)**	very narrow	Géant de Naples tardif	1
			narrow	Andes, Capvert	3
			medium	Broden, Lindon	5
			broad	Memphis, Vogue	7
			very broad	Torens	9
7.	**7.**	**VG**	**Leaf: ratio width/length**		
QN	**(*)**	**(a)**	compressed	Akita, Géant de Naples tardif	3
			medium	Astell, Buren	5
			elongated	Arbon, Lazio	7
8.	**8.**	**VG**	**Leaf: lobing**		
(+)		**(a)**	absent	Idol	1
QL			present	Atao, Romanesco ottobrino	9
9.	**9.**	**VG**	**Leaf: colour (with wax if present)**		
PQ		**(a)**	green	Baltimore, Belot, Lecerf	1
			grey green	Calisa, Géant de Naples tardif	2
			blue green	Arbon, Barrier Reef, Ciren	3
10.	**10.**	**VG**	**Leaf: intensity of colour (with wax if present)**		
QN	**(*)**	**(a)**	light	Baltimore, Ciren	3
			medium	Barrier Reef, Belot, Calisa	5
			dark	Arbon, Lecerf	7
11.	**11.**	**VG**	**Leaf: twisting of tip**		
QN		**(a)**	absent or very weak	Akita	1
			weak	Belot, Di Jesi	3
			medium	Barca, Imola	5
			strong	Oceano, Sernio	7
			very strong		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
12.	**12.**	**VG**	**Leaf: shape in cross-section**		
QN		**(a)**	concave	Bruce, Géant de Naples Tardif	1
			flat	Akita, Emeraude	2
			convex	Cortes	3
13.	**13.**	**VG**	**Leaf: blistering**		
QN		**(a)**	absent or very weak	Akita, Lecerf	1
			weak	Alpen, Opaal	3
			medium	Montano, Nautilus, Sergeant	5
			strong	Sernio, Siria	7
			very strong		9
14.	**14.**	**VG**	**Leaf: crimping near main vein**		
(+)		**(a)**	absent of very weak	Avelek, Fangio	1
QN			weak	Balmoral, Flanca	3
			medium	Mexico, Vinson	5
			strong	Akita, Sernio	7
			very strong	Izoar, Minioc	9
15.	**15.**	**VG**	**Leaf: undulation of margin**		
QN		**(a)**	absent of very weak	Etoile 23, Géant de Naples tardif	1
			weak	Akita, Beluga	3
			medium	Admirable, Alice Springs	5
			strong	Purdy, Siria	7
			very strong	Celebrity	9
16.	**16.**	**VG**	**Curd: covering by inner leaves**		
QN	**(*)**	**(b)**	not covered	Capvert, Opaal	1
			partly covered	Celesta, Eskimo	2
			fully covered	Amistad, Charif	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
17.	**17.**	**MS**	**Curd: height**		
(+)	**(*)**	**(b)**	short	Lecerf, Mechelse 2	3
QN			medium	Kernis, Tetris	5
			tall	Amistad, Gitano	7
18.	**18.**	**MS**	**Curd: diameter**		
QN	**(*)**	**(b)**	small	Lumina	3
			medium	Barrier Reef, Malaga	5
			large	Fremont, Novia, Plessi	7
19.	**19.**	**VG**	**Curd: shape in longitudinal section**		
(+)	**(*)**	**(b)**	circular	Gipsy Moth, Linero	1
PQ			transverse broad elliptic	Aviron, Melody	2
			transverse medium elliptic	Akita, Celesta	3
			transverse narrow elliptic	Erfurter, Lecerf	4
			triangular	Romanesco ottobrino	5
20.	**20.**	**VG**	**Excluding varieties with curd shape triangular: Curd: doming**		
(+)	**(*)**	**(b)**	weak	Burgh, Lecerf	3
QN			medium	Akita, Géant de Naples tardif	5
			strong	Belot, White Rock	7
21.	**21.**	**VG**	**Curd: colour**		
PQ	**(*)**	**(b)**	whitish	Astell, Iceberg	1
			yellow	Di Jesi	2
			orange	Cheddar, Sunset	3
			green	Amfora	4
G			violet	Graffiti	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
22.	**22.**	**VG**	**Curd: knobbling**		
(+)		**(b)**	very fine		1
QN			fine	Nautlilus, Opaal	3
			medium	Corvilia, Nedeleg	5
			coarse	Niagara	7
G			very coarse	Navona	9
23.	**23.**	**VG**	**Curd: texture**		
(+)		**(b)**	fine	Boris, Erfurter	3
QN			medium	Beluga, Gaviote	5
			coarse	Géant de Naples Tardif, Niagara	7
24.	**24.**	**VG**	**Curd: anthocyanin coloration after harvest maturity**		
QL			absent	Evita, Mantis	1
			present	Flanca, Planita	9
25. (+)	**25.**	**VG/MS**	**Flower: colour**		
QL	**(*)**		white	Bruce, Ecrin	1
G			yellow	Lecerf	2
26.	**26.**	**MS**	**Earliness in spring planting**		
(+)	**(*)**		very early		1
QN			very early to early		2
			early		3
			early to medium		4
			medium		5
			medium to late		6
			late		7
			late to very late		8
G			very late		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
27.	**27.**	**MS**	**Earliness in summer planting**		
(+)	**(*)**		very early autumn type		1
QN			very early to early autumn type		2
			early autumn type		3
			early to medium autumn type		4
			medium autumn type		5
			medium to late autumn type		6
			late autumn type		7
			late to very late autumn type		8
			very late autumn type		9
			very early winter type		10
			very early to early winter type		11
			early winter type		12
			early to medium winter type		13
			medium winter type		14
			medium to late winter type		15
			late winter type		16
			late to very late winter type		17
G			very late winter type		18
28.	**28.**	**VS/MS**	**Male sterility**		
(+)	**(*)**		absent	Alpha 2	1
QN			partial	Dunvez, Odegwen	2
G			total	Aviron, Bodilis	3

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 Explanations covering several characteristics

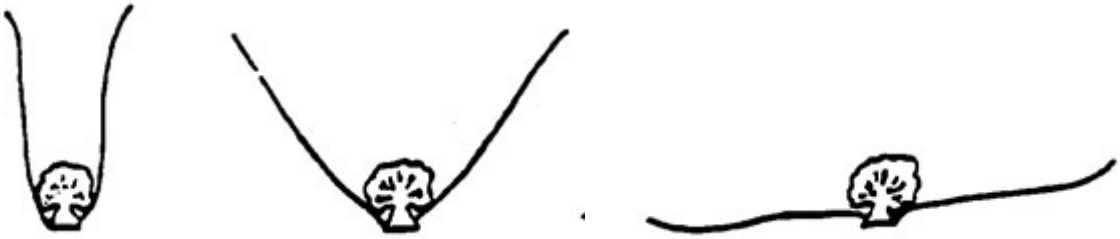
Characteristics containing the following key in the third column of the Table of Characteristics should be examined as indicated below:

a) Foliage and leaf: Observations on the foliage and the leaf which should be made at the time of full development of the foliage, before curd formation. All observations on the leaf should be made on the largest leaf.

b) Curd: Observations on the curd which should be made when the curd is fully developed (at harvest maturity).

8.2 Explanations for individual characteristics

Ad. 4: Leaf: attitude



1
erect

3
semi-erect

5
horizontal

Ad. 8: Leaf: lobing

1
absent

9
present

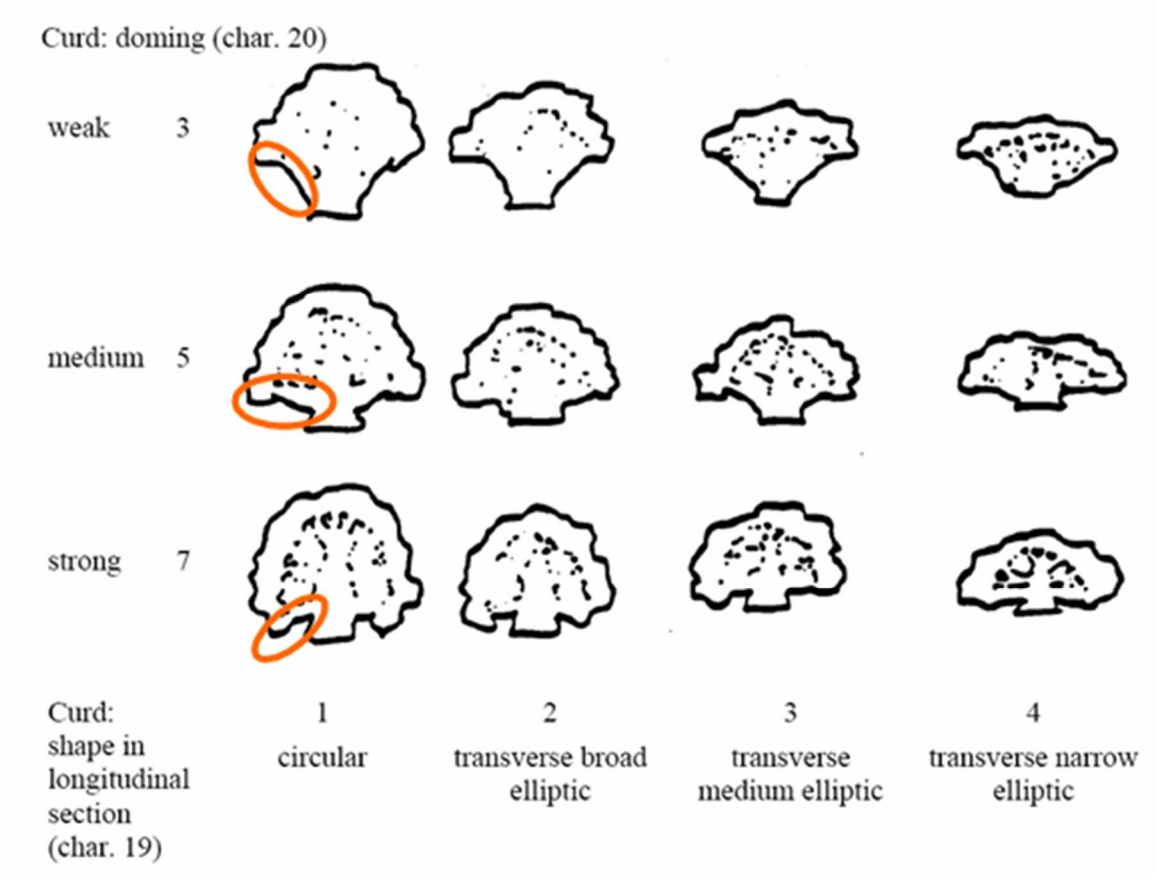
Ad. 14: Leaf: crimping near main vein

1	5	9
absent or very weak	medium	very strong

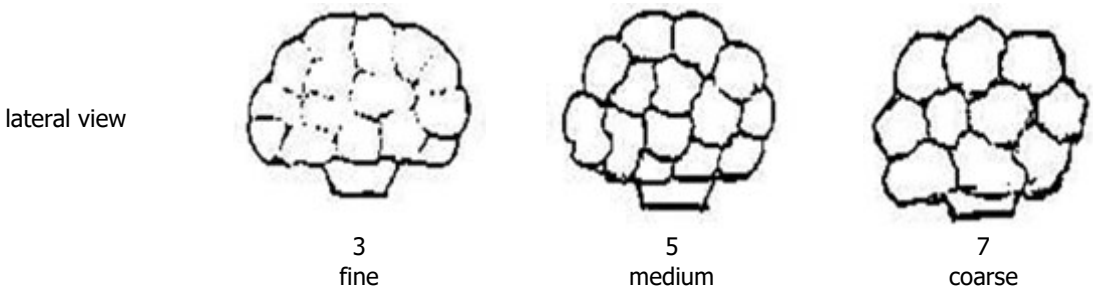
Ad. 17: Curd: height

3	5	7
short	medium	tall

Ad. 19: Curd: shape in longitudinal section
Ad. 20: Excluding varieties with curd shape: triangular: Curd: doming



Ad. 22: Curd: knobbling



Ad. 23: Curd: texture

The texture is "fine" when the surface of the curd is very smooth and is "coarse" when the surface of the curd is granular.

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:

Observation of color of flowers.

1	2
white	yellow

DNA marker test:

The markers are linked to the gene CCD4. The functional allele causes white petal color. Functional loss of this gene leads to yellow petal color. The markers corresponding with the functional or nonfunctional allele are based on 3 SNP markers located at position ~1296bp in the gene (Han et al. 2019).

The marker test can be performed in multiplex with the marker test for male sterility (Ad. 28).

The presence of the functional or nonfunctional CCD4 alleles 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, the variety has white flowers. Both probes are present (heterozygous), the variety has white flowers.
	Yellow (2)	Probe for nonfunctional CCD4 gene (yellow petal color) is homozygous present, the variety has yellow flowers. 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 white or yellow flowers due to another mechanism.

Ad. 26: Earliness in spring planting
Ad. 27: Earliness in summer planting

In cauliflower, earliness is strongly influenced by the temperature and the season of growing. Nevertheless, at the same place and for the same growing season, earliness is an important characteristic for the assessment of distinctness of varieties. For those reasons, no example varieties are provided in the Test Guidelines and the variety description should always state the place and the season of growing.

Ad. 28: Male sterility

To be tested in a field trial and/or in a DNA marker test[1].

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 (CMS))

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.

In cases where only a DNA marker test is allowed (state 1 and state 3 seed-propagated varieties), and the CMS marker 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 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.

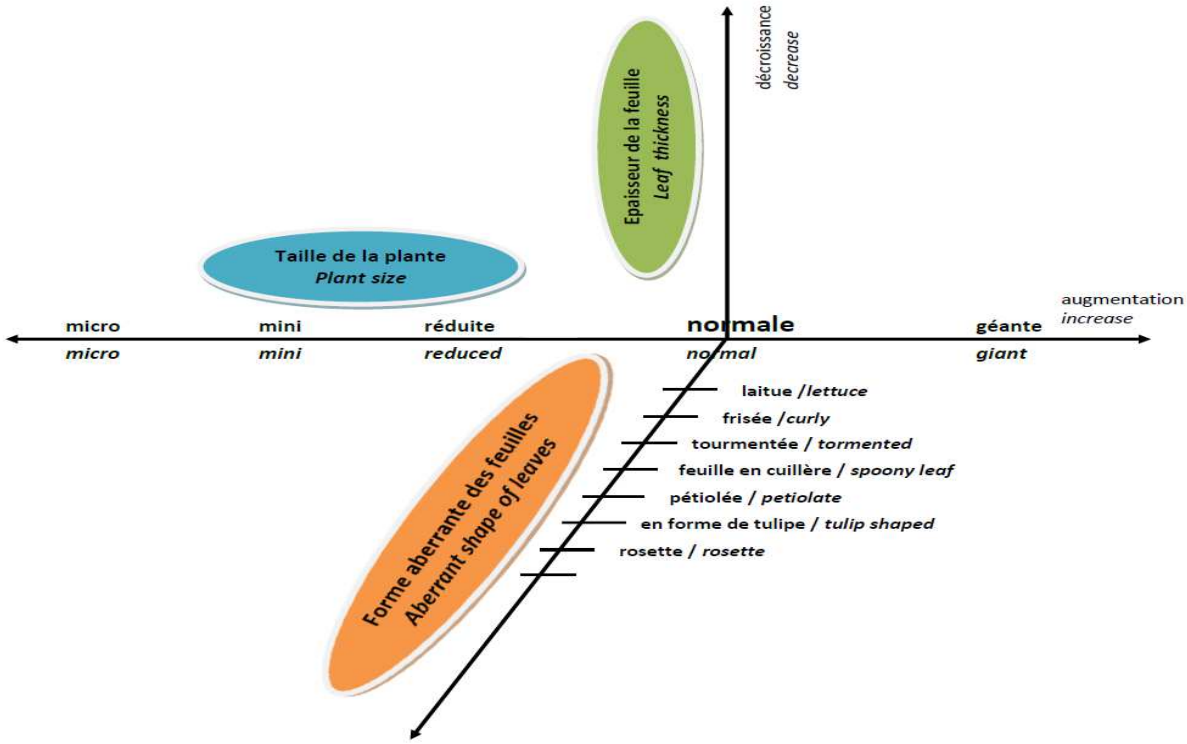
The marker test can be performed in multiplex with the marker test for flower color (Ad. 25).

[1] 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.

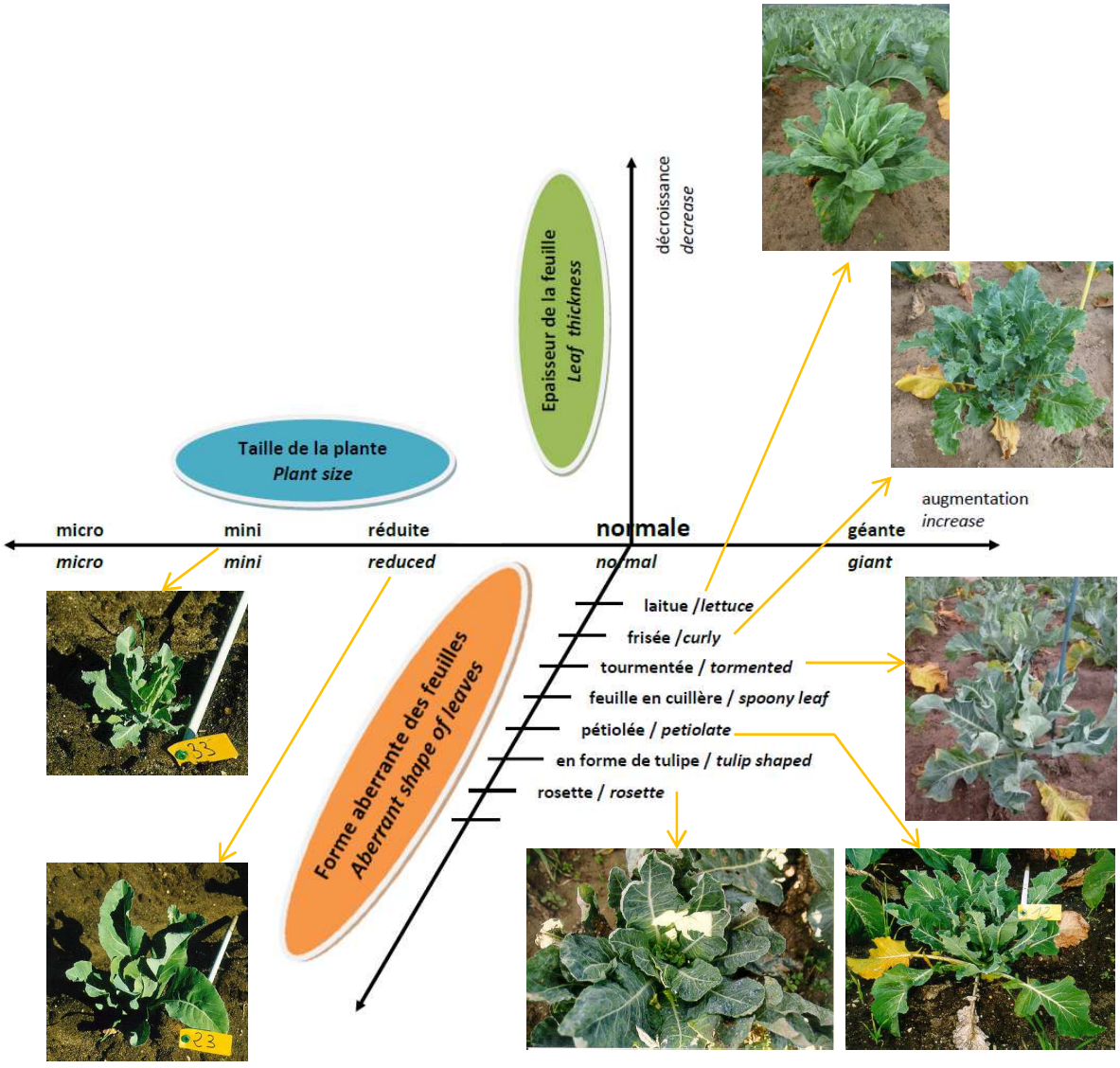
8.3 Aberrant plants

Phenotypes of aberrant plants are defined according to three criteria, which are expressed independently or simultaneously: the deformation of the vegetative system (curly leaves, tormented leaves, "salad" shape leaves...), a reduction of the vigour, and the thickening of the leaf blades.

The expression of abrerrance in a plant is independent of the observed genetic basis. That means that the same aberrant plant type could be observed in different varieties.



Main observed aberrant plant types



spatulée
spatulate

elliptique étroit
narrow elliptic

brillant
glossy

en gouttière
gutter shaped

asymétrique
asymetric

9. LITERATURE

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/045/2-Rev.3 – *Brassica oleracea* L. convar. *botrytis* (L.) Alef. Var. *botrytis* – cauliflower

Link to e-TQ:

https://online.plantvarieties.eu/backOfficeFormQuestions?viewFormId=14713&viewFormType=TQ&viewFormLang=EN&speciesName=brassica&status=1,2