



PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

***Brassica oleracea* L. var. *gemmifera* Zenker**

BRUSSELS SPROUT

UPOV Code: BRASS_OLE_GGM

Adopted on 28/01/2026

Entry into force on 15/03/2026

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CPVO-TP/054/2 Rev. 3

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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. var. *gemmifera* Zenker.

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/54/7 Rev. 2 dated 24/10/2023 (<https://www.upov.int/edocs/tgdocs/en/tg054.pdf>) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **15.03.2026**. 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.

3.4 Test design

3.4.1 Each test should be designed to result in a total of at least 40 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 of open pollinated and hybrid varieties (excluding single cross hybrids) should be according to the recommendations for cross-pollinated varieties in the UPOV-General Introduction to DUS.

For the assessment of uniformity of vegetatively propagated varieties, 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.

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) Plant: height (characteristic 1)
- b) Leaf blade: colour (characteristic 5)
- c) Leaf blade: intensity of colour (characteristic 6)
- d) Leaf blade: cupping (characteristic 8)
- e) Time of harvest maturity (characteristic 18)
- f) Male sterility (characteristic 20)

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.

Asterisked characteristics

In the case of disease resistance characteristics, only those resistances marked with an asterisk (*) in the CPVO column are compulsory.

6.2. 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
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.
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For column "stage, method":

MG, MS, VG, VS	– see Chapter 4.1.5
(a)	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/MG	Plant: height		
QN		(a)	very short		1
			very short to short		2
			short	Jade Cross	3
			short to medium		4
			medium	Cascade	5
			medium to tall		6
			tall	Bridge	7
			tall to very tall		8
G			very tall		9
2.	2.	VG	Plant: tendency to form a head		
QN		(a)	absent or very weak	Masterline	1
			very weak to weak		2
			weak	Cyrus	3
			weak to medium		4
			medium	Bridge	5
			medium to strong		6
			strong	Cor	7
			strong to very strong		8
			very strong	Oliver	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
3.	3.	VG	Leaf blade: size		
QN		(a)	very small		1
			very small to small		2
			small	Angus	3
			small to medium		4
			medium	Peer Gynt	5
			medium to large		6
			large	Braveheart	7
			large to very large		8
			very large		9
4.	4.	VG	Leaf blade: length		
QN		(a)	very short		1
			very short to short		2
			short	Prince Marvel	3
			short to medium		4
			medium	Cascade	5
			medium to long		6
			long	Braveheart	7
			long to very long		8
			very long		9
5.	5.	VG	Leaf blade: colour		
PQ		(a)	green	Masterline	1
			blue green	Angus	2
G			purple	Rubine	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6.	6.	VG	Leaf blade: intensity of colour		
QN		(a)	very light		1
			very light to light		2
			light	Origus, Prince Marvel	3
			light to medium		4
			medium	Angus, Boxer	5
			medium to dark		6
			dark	Estate, Placido, Rubine	7
			dark to very dark		8
G			very dark		9
7.	7.	VG	Leaf blade: waxiness		
QN		(a)	very weak		1
			very weak to weak		2
			weak	Evesham Special	3
			weak to medium		
			medium	Peer Gynt	5
			medium to strong		
			strong	Cavalier	7
			strong to very strong		
			very strong		

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
8.	8.	VG	Leaf blade: cupping		
QN		(a)	strongly convex		1
			strongly convex to moderately convex		2
			moderately convex		3
			moderately convex to plane		4
			plane	Braveheart	5
			plane to moderately concave		6
			moderately concave	Estate	7
			moderately concave to strongly concave		8
G			strongly concave	Explorer	9
9.	9.	VG	Leaf blade: blistering		
QN		(a)	very weak		1
			very weak to weak		2
			weak	Cavalier	3
			weak to medium		4
			medium	Masterline	5
			medium to strong		6
			strong	Breeze	7
			strong to very strong		8
			very strong		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
10.	11.	VG	Petiole: attitude		
QN		(a)	erect		1
			erect to semi-erect		2
			semi-erect	Montgomery	3
			semi-erect to horizontal		4
			horizontal	Angus	5
			horizontal to semi pendulous		6
			semi pendulous	Odessa	7
			semi pendulous to pendulous		8
			pendulous		9
11.	12.	VG	Petiole: length compared to blade		
QN		(a)	shorter		1
			shorter to moderately shorter		2
			moderately shorter	Braveheart	3
			moderately shorter to equal		4
			equal	Masterline	5
			equal to moderately longer		6
			moderately longer	Odessa	7
			moderately longer to longer		8
			longer		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
12.	13.	VG	Petiole: anthocyanin coloration		
QN	(a)		absent or very weak	Revenge	1
			very weak to weak		2
			weak	Breeze	3
			weak to medium		4
			medium	Odessa	5
			medium to strong		6
			strong	Prince Marvel	7
			strong to very strong		8
			very strong	Rasalon	9
13.	14.	VG	Sprout: shape in longitudinal section		
(+) PQ	(a)		narrow obovate	Explorer	1
			obovate		2
			broad obovate	Odessa	3
			circular	Braveheart	4
14.	15.	VG	Sprout: colour		
PQ	(a)		green	Estate	1
			blue green	Cascade	2
			purple	Rubine	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
15.	16.	VG	Sprout: intensity of colour		
QN		(a)	very light		1
			very light to light		2
			light	Prince Marvel	3
			light to medium		4
			medium	Estate	5
			medium to dark		6
			dark	Placido, Rubine	7
			dark to very dark		8
			very dark		9
16.	17.	VG	Sprout: density at harvest maturity		
QN		(a)	very loose		1
			very loose to loose		2
			loose	Steffiline	3
			loose to medium		4
			medium	Angus	5
			medium to dense		6
			dense	Prelent	7
			dense to very dense		8
			very dense		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
17.	18.	VG	Stem: spacing of sprout		
QN		(a)	very narrow		1
			very narrow to narrow		2
			narrow	Estate, Prelent	3
			narrow to medium		4
			medium	Cavalier, Cor	5
			medium to wide		6
			wide	Silverline	7
			wide to very wide		8
			very wide		9
18.	19.	MG/VG	Time of harvest maturity		
QN		(a)	very early	Lancer, Oliver	1
			very early to early		2
			early	Masterline, Peer Gynt	3
			early to medium		4
			medium	Lunet	5
			medium to late		6
			late	Braveheart, Bridge	7
			late to very late		8
G			very late	Ulysses	9
19.	20.	VG	Stem: profile of sprout column		
(+)		(a)	conical	Falstaff	1
QN			conical to cylindrical	Regent, Setterline	2
			cylindrical	Angus, Braveheart	3
20. (+)	21.	VS/MS	Male sterility		
QL		(a)	absent	Attis, Pontus	1
G			present	Abacus, Platinus	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
21. (+)	22.	VS	Resistance to <i>Plasmodiophora brassicae</i> (Pb) – Race Pb: 0		
QL			absent	Abacus	1
			present	Cryptus	9
22. (+)	23.	VS	Resistance to <i>Plasmodiophora brassicae</i> (Pb) – Race Pb: 1		
QL			absent	Abacus	1
			present	Cryptus	9
23. (+)	24.	VS	Resistance to <i>Plasmodiophora brassicae</i> (Pb) – Race Pb: 2		
QL			absent	Abacus, Cryptus	1
			present		9
24. (+)	25.	VS	Resistance to <i>Plasmodiophora brassicae</i> (Pb) – Race Pb: 3		
QL			absent	Abacus	1
			present	Cryptus	9

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

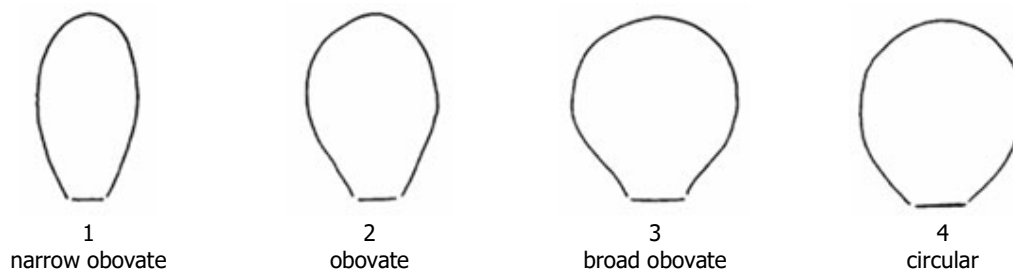
8.1 Explanations covering several characteristics

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

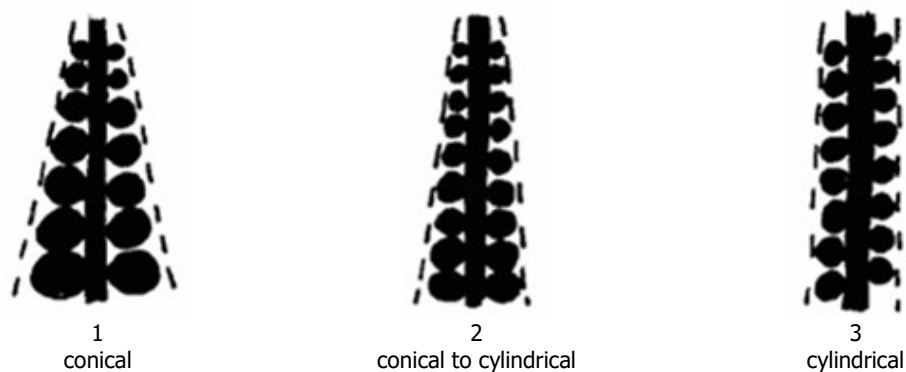
- a) All observations should be carried out at harvest maturity stage.

8.2 Explanations for individual characteristics

Ad. 13: Sprout/ shape in longitudinal section



Ad. 19: Stem: profile of sprout column



Ad. 20: Male sterility

To be tested in a field trial and/or in a DNA marker test¹.

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 coloured 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.



male fertile (pollen present)



male sterile (pollen absent)

DNA marker test:

If the cytoplasmic male sterility (CMS) marker is absent, the variety is expected to have male fertile flowers. If the CMS marker is present, the variety is expected to have male sterile 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 male fertile or male sterile flowers due to another mechanism.

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.

Ad. 21 to 24: Resistance to *Plasmodiophora brassicae* (Pb) – Races 0 to 3

1.	Pathogen	<i>Plasmodiophora brassicae</i>
2.	Quarantine status	no
3.	Host species	<i>Brassica oleracea</i>
4.	Source of inoculum	Naktuinbouw ² (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 <i>Brassica oleracea</i> 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) ⁴ 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 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	Pipette 1 ml on both sides at the base of each seedling, totalling 2 ml per plant.
10.7	Observation, evaluation and end of test	6 weeks after inoculation (destructive)

² Naktuinbouw: resistentie@naktuinbouw.nl

³ 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	Observation scale	class 0 = no galling class 1 = a few small galls class 2 = 2a or 2b (2a = moderate galling; 2b = slight swelling of the main root and browning and ultimately death of all lateral roots) class 3 = severe galling
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.



class 0



class 1



class 2a



class 2b



class 3

9. LITERATURE

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO/TQ-054/2 Rev.3 – *Brassica oleracea* L. var. *gemmifera* Zenker – Brussels sprout

Link to e-TQ:

<https://online.plantvarieties.eu/backOfficeFormQuestions?viewFormId=17737&viewFormType=TQ&viewFormLanguage=EN&commonName=Brussels&type=2&status=2,1&order=formName>