



PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Brassica oleracea L. var. *sabellica* L.

CURLY KALE

UPOV Code: BRASS_OLE_GAS

Adopted on 16/02/2011

Entered into force on 01/01/2011

I SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on general UPOV Document TG/1/3 and UPOV Guideline TG/90/6 dated 31/03/2004 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to varieties of *Brassica oleracea* L. var. *sabellica* L.

II SUBMISSION OF SEED AND OTHER PLANT MATERIAL

1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of

- the closing date for the receipt of plant material;
- the minimum amount and quality of plant material required;
- the examination office to which material is to be sent.

A sub-sample of the material submitted for test will be held in the variety collection as the definitive sample of the candidate variety.

The applicant is responsible for ensuring compliance with any customs and plant health requirements.

2. Final dates for receipt of documentation and material by the Examination Office

The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen.

The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO whether acceptable plant material has been received or not. However if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Plant material requirements

The current quality and quantity requirements as well as the final dates for submission of the plant material are available on the CPVO website (www.cpvo.europa.eu).

Quality of seed: Should not be less than the standards laid down for certified seed in Annex II of Council Directive 2002/55/EC.

Seed Treatment: The plant material must not have undergone any treatment unless the CPVO and the Examination Office allow or request such treatment. If it has been treated, full details of the treatment must be given.

Special requirements: -

Labelling of sample: - Species
- File number of the application allocated by the CPVO
- Breeder's reference
- Examination reference (if known)
- Name of applicant
- The phrase "On request of the CPVO"

III CONDUCT OF TESTS

1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation (EC) No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;
- varieties protected in other UPOV Member States;
- any other variety in common knowledge.

The composition of the variety collection in each Examination Office depends on the environmental conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

2. Material to be examined

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the variety collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate the work with other Offices involved in DUS testing of curly kale. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

3. Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the Annex 1. 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. In the latter case, the CPVO should be informed. In addition the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation (EC) No.874/2009, to insert additional characteristics and their expressions in respect of a variety.

4. Grouping of varieties

The varieties and candidates to be compared will be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups. The characteristics used for grouping could be the following:

- a) Plant: height (characteristic 1)
- b) Leaf: anthocyanin coloration (characteristic 5)
- c) Leaf: colour of fully developed leaf (characteristic 8)
- d) Leaf blade: density of "curling" (characteristic 14)

5. Trial designs and growing conditions

The minimum duration of tests will normally be two independent growing cycles. Tests will be carried out under conditions ensuring normal growth. The size of the plots will be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing period.

The test design is as follows:

As a minimum, each test should include a total of 60 plants, divided between two or more replicates.

All observations determined by measurement or counting should be made on 20 plants or parts of 20 plants.

6. Special tests

In accordance with Article 83(3) of Council Regulation (EC) 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, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special 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.

7. Standards for decisions

a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of Article 7 of Council Regulation (EC) No. 2100/94.

b) **Uniformity**

For the assessment of uniformity of:

- (i) Cross pollinated and hybrid varieties (excluding single cross hybrids), relative uniformity standards should be applied
- (ii) 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.

Table of maximum number of off-types allowed for uniformity standards for vegetatively propagated varieties, single cross hybrids and inbred lines

Number of plants	Off-types allowed
6-35	1
36-82	2

c) **Stability**

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity.

IV REPORTING OF RESULTS

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two growing periods but in some cases three growing periods may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive interim reports and final reports by the date agreed between the CPVO and the examination office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

V LIAISON WITH THE APPLICANT

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 agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

The interim report as well as the final report shall be sent by the Examination Office to the CPVO.

VI ENTRY INTO FORCE

The present protocol enters into force on **01/01/2011**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the new TP. 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 the submission of plant material for the first growing period.

In cases where the CPVO requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process of being carried out at the moment of the 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.

ANNEXES TO FOLLOW

ANNEX I

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Legend:

Note: For the CPVO numbered characteristics, all characteristics in the table are compulsory; notwithstanding, in the case of disease resistance characteristics, only those resistances marked with an asterisk (*) in the CPVO column are compulsory. The asterisks in the UPOV numbered characteristics are there for information purposes and denote those characteristics which should always be observed when a UPOV guideline is utilised.

In general for the assessment of resistance characteristics, the facilities of other Examination Offices or specialised institutions might be used, subject to previous arrangements.

Some characteristics may be discarded: if there are already phytosanitary restrictions.

- (+) See explanations on the Table of characteristics
- (a) See explanations on the table of characteristics
- G Grouping characteristic

Types of expression of characteristics:

- QL Qualitative characteristic
- QN Quantitative characteristic
- PQ Pseudo-qualitative characteristic

Type of 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

When a method of observation is attributed to a certain characteristic, the first differentiation is made depending if the action taken is a visual observation (V) or a measurement (M).

The second differentiation deals with the number of observations the expert attributes to each variety, thus the attribution of either G or S.

If a single observation of a group consisting of an undefined number of individual plants is appropriate to assess the expression of a variety, we talk about a visual observation or a measurement made on a group of plants, thus we attribute the letter G (either VG or MG). If the expert makes more than one observation on that group of plants, the decisive part is that we have at the end only one data entry per variety which means that we have to deal with G (e.g. measurement of plant length on a plot – MG, visual observation of green colour of leaves on a plot – VG).

If it is necessary to observe a number of individual plants to assess the expression of a variety, we should attribute the letter S (thus either VS or MS). Single plant data entries are kept per variety for further calculations like the variety mean (e.g. measurement of length of ears – MS, visual observation of growth habit of single plants in grasses – VS). The number of individual plants to be observed in such cases is stated in section III.5.

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ANNEX II

Technical Questionnaire

ANNEX I

TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTIONS

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note
1. QN G	1. (*)	VG/MG	Plant: height		
		220-240	short	Niedriger grüner krauser	3
			medium	Frosty, Hammer	5
			tall	Westlandse Herfst	7
2. QN	2.	VG/MG	Plant: diameter		
		220-240	small		3
			medium	Spurt	5
			large	Hammer	7
3. PQ (+)	3. (*) (+)	VG	Plant: shape (fully developed plants)		
		140-240	inverted pyramid	Lerchenzungen	1
			flat	Kobolt	2
			dome	Nero di Toscana	3
			pyramid		4
			column	Arsis, Westlandse Herfst	5
4. (+) QN	4. (+)	VG	<u>Only varieties of dome, pyramid or column shape:</u> Plant: position of growing point in relation to top of plant		
		140-180	same level	Pentland Brig, Lav opretvoksende	1
			slightly below	Spurt	3
			deeply below	Vates	5
5. QL G	5. (*)	VG	Leaf: anthocyanin coloration		
		60-220	absent	Lerchenzungen, Pentland Brig	1
		(a)	present	Garna Red	9
6. PQ	6.	VG	Leaf: distribution of anthocyanin coloration		
		140-180	partial	Cottagers	1
		(a)	entire leaf	Garna Red, Redbor	2

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note			
7.	7.	VG	Leaf: colour of <u>young</u> leaf					
			PQ	140-180	yellow green	Frosty, Hammer	1	
					(a)	green	Dwarf Green Curled	2
					grey green	Lerchenzungen	3	
					blue green	Vates	4	
red or purple	Garna Red	5						
8.	8.	VG	Leaf: colour of <u>fully developed</u> leaf					
			PQ	140-180	yellow green	Hammer	1	
					(a)	green	Frosty	2
					grey green	Lerchenzungen	3	
					blue green	Vates	4	
G		red or purple	Garna Red	5				
9.	9.	VG	Leaf: intensity of colour of <u>fully developed</u> leaf					
			QN	140-180	light		3	
					(a)	medium		5
dark		7						
10.	10.	VG	Leaf blade: shape					
			PQ	140-180	very narrow elliptic	Lerchenzungen	1	
					(a)	very narrow elliptic to narrow elliptic	Kobolt	2
					narrow elliptic	Hammer	3	
					narrow elliptic to elliptic	Frosty, Halbhoher grüner krauser	4	
elliptic	Westlandse Herfst	5						
11.	11.	VG/MS	Leaf blade: length					
			QN	140-180	short	Vates	3	
					(a)	medium	Spurt	5
long	Lerchenzungen	7						

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note
12.	12.	VG/MS	Leaf blade: width		
QN	(*)	140-180	narrow	Vates	3
		(a)	medium	Spurt	5
			broad	Westlandse Herfst	7
13.	13.	VG	Leaf blade: curvature of midrib		
QN	(+)	140-180	weak	Lerchenzungen	3
		(a)	medium	Hammer	5
			strong	Halbhoher grüner krauser	7
14.	14.	VG	Leaf blade: density of "curling" (leaves at middle of plant)		
QN	(*)	140-180	absent or very low	Cottagers	1
(+)	(+)	(a)	low	Garna Red, Pentland Brig	3
			medium	Dwarf Green Curled	5
G			high	Halbhoher grüner krauser, Westlandse Herfst	7
15.	15.	VG	Leaf blade: folding in cross section		
QN		140-180	weak	Pentland Brig	3
(+)	(+)	(a)	medium	Vates	5
			strong	Lerchenzungen	7
16.	16.	VG	Petiole: attitude at middle of plant		
QN	(*)	140-180	erect	Arsis	1
			semi-erect	Vates	3
			horizontal	Kobolt	5
17.	17.	VG/MS	Petiole: length		
QN		180-220	short		3
			medium	Spurt	5
			long	Halbhoher grüner krauser	7

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note
18.	18.	VG/MS	Petiole: width		
QN		180-220	narrow	Hammer	3
			medium	Halbhoher grüner krauser	5
			broad		7

EXPLANATIONS AND METHODS

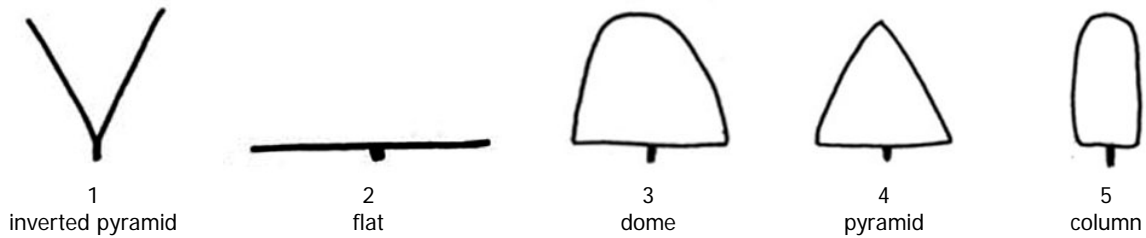
1 Explanations covering several characteristics

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

- (a) Leaf: Observations on the foliage and the leaf which should be made at on fully developed leaves which show no sign of senescence.

2 Explanations for individual characteristics

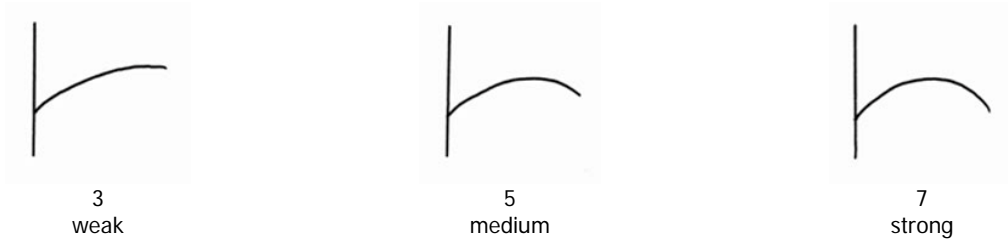
Ad 3: Plant: shape (fully developed plants)



Ad 4: Plant: position of growing point in relation to the top of plant



Ad 13: Leaf blade: curvature of midrib



Ad 14: Leaf blade: density of "curling" (leaves at middle of plant)



1
absent or very low



3
low



5
medium



7
high

Ad 15: Leaf blade: folding in cross section



3
weak



5
medium



7
strong

Key for growth stage (Growth key):

00	dry seed
10	germination
15	fully opened cotyledons
20	early growth of first true leaf
25	early growth of second true leaf
30	first true leaf fully developed
40	second true leaf fully developed
50	third true leaf fully developed
60	fourth true leaf fully developed
100	new leaves developing rapidly
110	early stem formation
140	plant developing mature shape
160	lower leaves becoming coarse and large
180	middle leaves well developed, but not too coarse
200	stem fully developed becoming woody
220	plant fully developed with mature shape
240	lower leaves beginning to senesce
260	leaves at lower and middle part of plant senescing
280	very slow development of new leaves
400	initiation of flowering

LITERATURE

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Langer, R.H.M., and Hill, G.D., 1982: "Agricultural Plants 8, Cruciferae", 165-183, Cambridge University Press, Cambridge.

Lustinec, J., 1988: "III. 11 Kale (*Brassica oleracea* L. var. *acephala*, *medullosa*, *ramosa*, *sabellica*)", 530-547, in: Biotechnology in Agriculture and Forestry 6. Ed: Y.P.S. Bajaj, Springer-Verlag Berlin.

Nieuwhof, M., 1969: "Cole Crops: Botany, Cultivation and Utilisation", Leonard Hill, London.

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

ANNEX II



TECHNICAL QUESTIONNAIRE

to be completed in connection with an application for Community Plant Variety Rights
Please answer all questions. A question without any answer will lead to a non-attribution
of an application date. In cases where a field / question is not applicable, please state so.

1. **Botanical taxon:** Name of the genus, species or sub-species to which the variety belongs and common name

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2. **Applicant(s):** Name(s) and address(es), phone and fax number(s), Email address, and where appropriate name and address of the procedural representative

3. **Variety denomination**

a) Where appropriate proposal for a variety denomination:

b) Provisional designation (breeder's reference):

4. Information on origin, maintenance and reproduction of the variety

4.1 Breeding, maintenance and reproduction of the variety

Please indicate breeding scheme, parents, other relevant information

- (a)
 - (i) hybrid []
 - (ii) open-pollinated variety..... []
 - (iii) parent line []

- (b)
 - (i) seed propagated []
 - (ii) vegetatively propagated..... []

- (c) Other information on genetic origin and breeding method []

4.2 Method of propagating the variety

- (a) Seed propagated varieties
 - (i) Cross-pollination []
 - (ii) Hybrid
 - seed-propagated parents []
 - one vegetatively propagated and one seed-propagated parent..... []
 - two vegetatively propagated parents []
 - (iii) Other (please provide details) []

- (b) Vegetative propagated varieties
 - (i) cuttings []
 - (ii) *in vitro* propagation..... []
 - (iii) other (state method) []

4.3 Geographical origin of the variety: the region and the country in which the variety was bred or discovered and developed

<p>4.4 Shall the information on data relating to components of hybrid varieties including data related to their cultivation be treated as confidential?</p> <p>[] YES [] NO</p> <p>If yes, please give this information on the attached form for confidential information.</p> <p>If no, please give information on data relating to components of hybrid varieties including data related to their cultivation:</p> <p>Breeding scheme (indicate female component first)</p>		
<p>5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in the CPVO Protocol; please mark the state of expression which best corresponds).</p>		
Characteristics	Example varieties	Note
<p>5.1 (1) Plant: height</p> <p>short</p> <p>medium</p> <p>tall</p>	<p>Niedriger grüner krauser</p> <p>Frosty, Hammer</p> <p>Westlandse Herfst</p>	<p>3 []</p> <p>5 []</p> <p>7 []</p>
<p>5.2 (5) Leaf: anthocyanin coloration</p> <p>absent</p> <p>present</p>	<p>Lerchenzungen, Pentland Brig</p> <p>Garna Red</p>	<p>1 []</p> <p>9 []</p>
<p>5.3 (8) Leaf: colour of <u>fully developed</u> leaf</p> <p>yellow green</p> <p>green</p> <p>grey green</p> <p>blue green</p> <p>red of purple</p>	<p>Hammer</p> <p>Frosty</p> <p>Lerchenzungen</p> <p>Vates</p> <p>Garna Red</p>	<p>1 []</p> <p>2 []</p> <p>3 []</p> <p>4 []</p> <p>5 []</p>
<p>5.4 (11) Leaf blade: length</p> <p>short</p> <p>medium</p> <p>long</p>	<p>Vates</p> <p>Spurt</p> <p>Lerchenzungen</p>	<p>3 []</p> <p>5 []</p> <p>7 []</p>

Characteristics		Example varieties	Note
5.5 (12)	Leaf blade: width		
	narrow	Vates	3 []
	medium	Spurt	5 []
	broad	Westlandse Herfst	7 []
5.6 (14)	Leaf blade: density of "curling" (leaves at middle of plant)		
	absent or very low	Cottagers	1 []
	low	Garna Red, Pentland Brig	3 []
	medium	Dwarf Green Curled	5 []
	high	Halbhoher grüner krauser, Westlandse Herfst	7 []
5.7 (16)	Petiole: attitude at middle of plant		
	erect	Arsis	1 []
	semi-erect	Vates	3 []
	horizontal	Kobolt	5 []
6. Similar varieties and differences from these varieties:			
Denomination of similar variety	Characteristic in which the similar variety is different ¹⁾	State of expression of similar variety	State of expression of candidate variety
<p>¹⁾ In the case of identical states of expressions of both varieties, please indicate the size of the difference</p>			
7. Additional information which may help to distinguish the variety			
7.1 Resistance to pests and diseases			

7.2 Special conditions for the examination of the variety

YES, please specify

NO

7.3 Other information

YES, please specify

NO

8. GMO-information required

The variety represents a Genetically Modified Organism within the meaning of Article 2(2) of Council Directive EC/2001/18 of 12/03/2001.

YES NO

If yes, please add a copy of the written attestation of the responsible authorities stating that a technical examination of the variety under Articles 55 and 56 of the Basic Regulation (EC) No. 2100/94 does not pose risks to the environment according to the norms of the above-mentioned Directive.

9. Information on plant material to be examined

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 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 the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

- | | | |
|---|------------------------------|-----------------------------|
| (a) Microorganisms (e.g. virus, bacteria, phytoplasma) | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| (b) Chemical treatment (e.g. growth retardant or pesticide) | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| (c) Tissue culture | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| (d) Other factors | <input type="checkbox"/> Yes | <input type="checkbox"/> No |

Please provide details of where you have indicated "Yes":

10. Possible place of the technical examination

In case the CPVO needs to arrange a technical examination for this candidate variety, there might be more than one examination office entrusted by the CPVO suitable to grow your variety. In this case, the Office will decide on the place of the technical examination but you might wish to express here a preference in respect of an examination office. The available entrusted examination offices for that species can be found in the S2 Gazette under <http://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette>

I/we hereby declare that to the best of my/our knowledge the information given in this form is complete and correct.

Date

Signature

Name

[End of document]