



PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

***Valerianella locusta* L.
& *Valerianella eriocarpa* Desv.**

CORNSALAD

UPOV Code: VLRNL_LOC; VLRNL_ERI

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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 *Valerianella locusta* L. and *Valerianella eriocarpa* Desv.

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/75/7 Rev. dated 24/10/2023 (<https://www.upov.int/edocs/tgdocs/en/tg075.pdf>) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **01.01.2024**. 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 growing cycle.

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 four weeks after the date of the request for technical examination by the CPVO and in any case preferably before the submission period of the plant material.

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

In cases where the Examination Office identifies issues during the course of the technical examination that may lead to a negative report, the Examination Office shall inform the CPVO and in urgent cases the applicant/holder as soon as such issues become obvious.

1.3.3 Sample keeping in case of problems

As far as feasible the Examination Office shall keep a representative sample of any relevant testing material of the candidate variety and reference variety(ies) if the technical examination has resulted in a negative report. As soon as possible, the CPVO shall inform the Examination Office when the material can be destroyed.

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/S3 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 submission of plant 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 in writing.

3. METHOD OF EXAMINATION

3.1 Number of growing cycles

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

The testing of a variety may be concluded when the entrusted examination office can determine with certainty the outcome of the test.

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/tqpdocs/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 Special tests for additional characteristics

In accordance with Article 23 of Implementing Rules N° 874/2009 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 characteristics 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 common knowledge varieties that are suitable to grow in the 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 include varieties protected under National 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.

The inventory shall take into account the list of varieties which are the subject of an on-going application for protection or official registration (candidate varieties).

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.

Living material in variety collections representing varieties for which a DUS test was carried out at that EO shall be renewed after verification in a side-by-side comparison. In case where no living material is available anymore in the collection, such verification could be done with any other test that has proven to give similar results 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.

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**

4.2.1 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 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:

4.2.2 This Technical Protocol has been developed for the examination of seed propagated varieties. For varieties with other types of propagation the recommendations in the UPOV-General Introduction to DUS and document TGP/13 "Guidance for new types and species", Section 4.5 "Testing Uniformity" should be followed.

For the assessment of uniformity of self-pollinated seed-propagated varieties, 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.

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 ORGANISATION 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 organise the growing trial so that similar varieties are grouped together.
- 5.3** The following have been agreed as useful grouping characteristics:
- a) Leaf: length (characteristic 3)
 - b) Leaf: profile of apical part in longitudinal section (characteristic 9)
 - c) Seed: size (characteristic 19)
 - d) Seed: collar (characteristic 20)
- 5.4** If characteristics other than those mentioned in the list of grouping characteristics and/or from the table of characteristics and/or from the Technical Questionnaire – sections 5 and 7 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.
- 5.5** Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the UPOV-General Introduction to DUS and document TGP/9 “Examining Distinctness”.

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

States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description. All relevant states of expression are presented in the characteristic.

Further explanation of the presentation of states of expression and notes is provided in UPOV document TGP/7 “Development of Test Guidelines”.

6.3 Example Varieties

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

6.4 Legend

For column 'CPVO N°':

G	Grouping characteristic	-see Chapter 5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	Explanations for individual characteristics	-see Chapter 8.2
(*)	Asterisked characteristic	-see Chapter 6.1

For column 'UPOV N°':

The numbering of the characteristics is provided as a reference to the UPOV guideline.

(*)	UPOV Asterisked characteristic	- Characteristics that are important for the international harmonization of variety descriptions.
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For column 'Stage, method':

MG, MS, VG, VS	-see Chapter 4.1.5
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7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1.	1.	VG	Plant: attitude		
	(*)				
QN			erect	Elan	1
			erect to semi-erect		2
			semi-erect	Verte de Louviers	3
			semi-erect to horizontal		4
			horizontal	Valgros	5
2.	2.	VG	Plant: diameter		
	(*)				
QN			very small		1
			very small to small		2
			small	Coquille de Louviers	3
			small to medium		4
			medium	Verte de Louviers	5
			medium to large		6
			large	Verte de Cambrai	7
			large to very large		8
			very large	A grosse graine	9
3.	3.	MS	Leaf: length		
	(*)				
QN			very short		1
			very short to short		2
			short	Coquille de Louviers	3
			short to medium	Verte de Cambrai	4
			medium	Verte à coeur plein 2	5
			medium to long	Arpege	6
			long	Agathe	7
			long to very long	A grosse graine	8
G			very long	Ljubljanski	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
4. QN	4. (*)	MS	Leaf: width		
			very narrow		1
			very narrow to narrow		2
			narrow	Verte d'Etampes	3
			narrow to medium		4
			medium	A grosse graine, Verte de Cambrai	5
			medium to broad		6
			broad	Palace, Rodion	7
			broad to very broad		8
very broad		9			
5. QN	5.	MS	Leaf: ratio length/width		
			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. (+) PQ	6. (*)	VG	Leaf: shape		
			elliptic	Verte de Louviers	1
			broad spatulate	Verte à coeur plein 2	2
			narrow spatulate	A grosse graine	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
7. QN	7.	VG	Leaf: glossiness		
			very weak		1
			very weak to weak		2
			weak	D'Italie à feuille de laitue	3
			weak to medium		4
			medium	Verte maraîchère	5
			medium to strong		6
			strong	Verte de Louviers	7
			strong to very strong		8
			very strong		9
8. QN	8.	VG	Leaf: profile in cross-section		
			concave		1
			flat	Coquille de Louviers	2
			convex	Verte à coeur plein 2	3
9. (+) QN	9. (*)	VG	Leaf: profile of apical part in longitudinal section		
			concave	Coquille de Louviers	1
			flat	Gala, Verte à coeur plein 2	2
G			convex	Verte d'Étampes	3
10. QN	10.	VG	Leaf: torsion		
			absent or very weak		1
			very weak to weak		2
			weak	Dante	3
			weak to medium		4
			medium	A grosse graine	5
			medium to strong		6
			strong	Topaze	7
			strong to very strong		8
			very strong		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
11. QN	11. (*)	VG	Leaf: intensity of green colour		
			very light		1
			very light to light		2
			light	Verte maraîchère	3
			light to medium		4
			medium	Verte de Rouen	5
			medium to dark		6
			dark	Verte à coeur plein 2	7
			dark to very dark		8
		very dark		9	
12. QL	12.	VG	Leaf: dentation (outer leaves)		
			absent	A grosse graine, Coquille de Louviers	1
			present	Saphir, Sapiana	9
13. QN	13.	VG	Leaf: thickness		
			very thin		1
			very thin to thin		2
			thin	Valgros	3
			thin to medium		4
			medium		5
			medium to thick		6
			thick	Verte d'Etampes	7
			thick to very thick		8
		very thick		9	

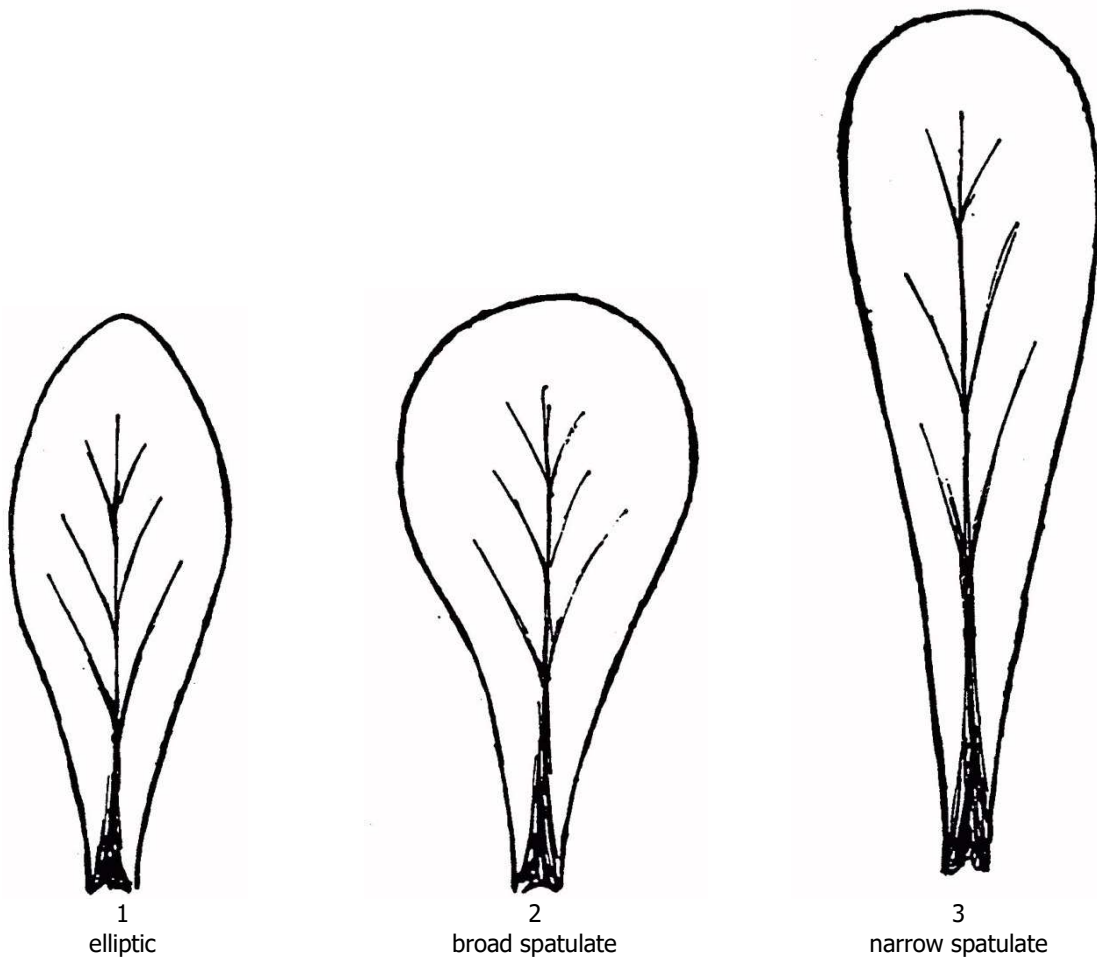
CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
14.	14.	VG	Leaf: prominence of veins		
QN	(*)		absent or very weak		1
			very weak to weak		2
			weak	Verte de Louviers	3
			weak to medium		4
			medium	Progres	5
			medium to strong		6
			strong	Toendra, Vit	7
			strong to very strong		8
			very strong		9
15.	15.	VG	Leaf: blistering		
QN			absent or very weak	A grosse graine, Baron	1
			very weak to weak		2
			weak		3
			weak to medium		4
			medium	D'Italie à feuille de laitue, Saphir	5
			medium to strong		6
			strong	Progres	7
			strong to very strong		8
			very strong		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
16.	16.	MG	Time of beginning of bolting (10% of plants)		
QN			very early	Valgros	1
			very early to early		2
			early	Verte à coeur plein 2	3
			early to medium		4
			medium	Verte d'Etampes	5
			medium to late		6
			late	Baikal	7
			late to very late		8
			very late		9
17.	17.	VG	Flower stem: fasciation		
QL			absent	A grosse graine, Coquille de Louviers	1
			present	Jobra, Jovis	9
18.	18.	VG	Flower stem: anthocyanin coloration		
QN			very weak		1
			very weak to weak		2
			weak	A grosse graine	3
			weak to medium		4
			medium	Valvert	5
			medium to strong		6
			strong	Pustade	7
			strong to very strong		8
			very strong		9

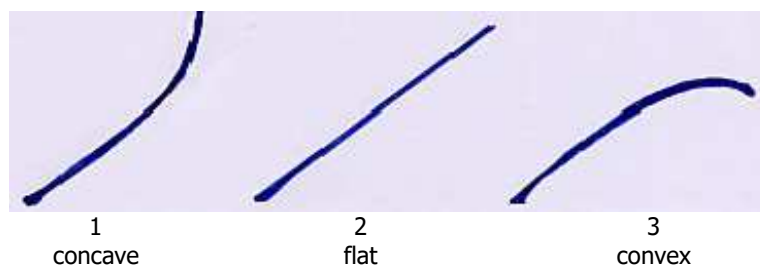
CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
19. QN G	19. (*)	VG	Seed: size		
			very small		1
			very small to small		2
			small	D'Italie à feuille de laitue, Deutscher	3
			small to medium		4
			medium	Vit	5
			medium to large		6
			large	A grosse graine	7
			large to very large		8
			very large		9
20. (+) QL G	20. (*)	VG	Seed: collar		
			absent	Deutscher	1
			present	D'Italie à feuille de laitue	9
21. (+) QL 21.1 21.2	21.	VG	Resistance to downy mildew (<i>Peronospora valerianella</i>)		
			Stain 1		
			absent		1
			present		9
			Stain 2		
			absent	Deutscher	1
present	D'Italie à feuille de laitue	9			

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

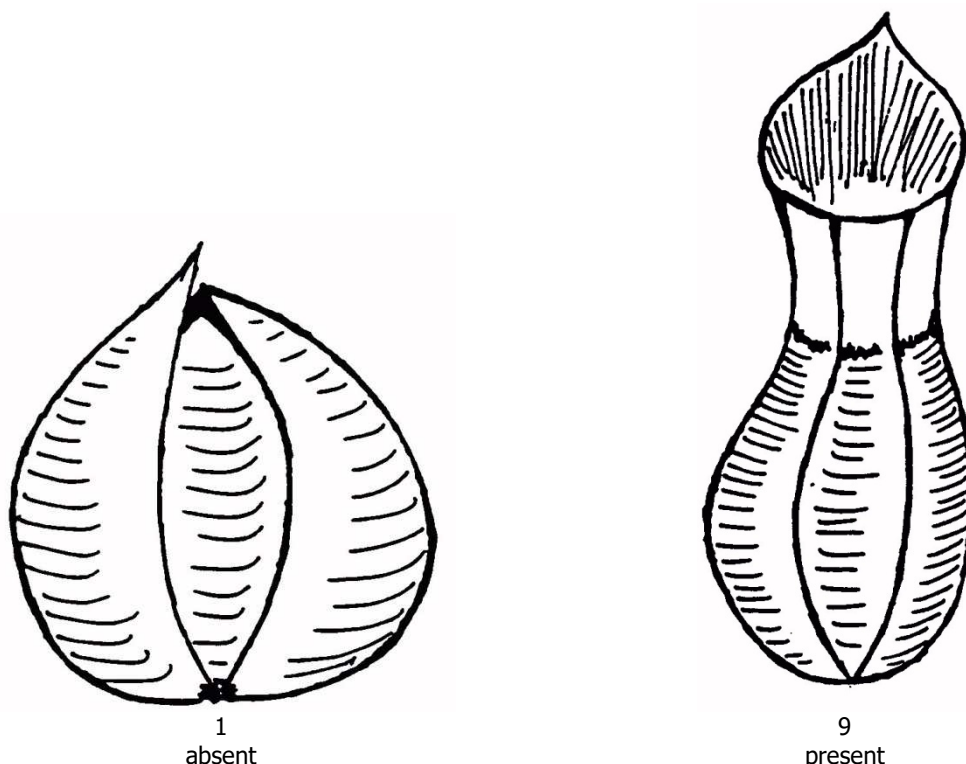
Ad. 6: Leaf: shape



Ad. 9: Leaf: profile of apical part in longitudinal section



Ad. 20: Seed: collar



Ad. 21: Resistance to downy mildew (*Peronospora valerianella*)

Strain(s) used: Races 1, 2

Maintenance of strains:

Nature of medium: Conservation of strains in the form of oospores associated with naturally contaminated seeds.

Particular conditions: Storage of batches of contaminated seeds in waterproof sachets at 4°C, on different varieties.

Comments: Since mildew spores are fragile, medium-term conservation (a few months) on contaminated leaves kept in a freezer (-20°C) is particularly delicate.

Production of inoculum:

At the beginning of the test period, inoculum is produced from naturally contaminated seeds (sanitary analysis). Broadcast sowing, about 200-250 seeds per box. Germination and development of plant germs in a cold tunnel, 10 to 15°C.

From the time of appearance of the first leaf, the boxes are placed in a plastic cage or are covered with a plastic lid (mini-glasshouse), in order to generate moisture on the plants.

10 to 12 days after sowing, the first symptoms appear on the plants produced from the infected seeds. The cotyledons and leaves have a rolled aspect. The sick leaves are recovered in order to multiply the inoculum or for an infection. The spores are collected on a recent sporulation (night time). They are suspended in a small amount of permuted water, with 20 tween added (1 drop per 100 ml) and filtered on stamens. The concentration in spores is adjusted to 10⁵ spores/ml. The inoculum is kept on a bed of ice.

Sowing:

Sowing in plugs (5 x 5 cm), at a rate of 2 to 3 seeds per plug, in order to conserve only one seed per plug.

Conduct of the test:

Plant stage: First leaf stage

Number of plants studied: 40 plants per variety and 10 plants of a control variety.

Cultivation conditions: 10 to 15°C before inoculation / 8 to 15°C after inoculation: the difference in temperature is important.
Implantation: Cold glasshouse prior to inoculation / cold tunnel (anti-freeze) after inoculation.
Inoculation: Spraying of a suspension of spores at 10⁵ spores/ml, using an ECOSPRAY type sprayer, then the plants are covered for 48 hours in a plastic cage.
Duration of the test: Sowing-inoculation: approximately 10-12 days
Inoculation-reading: 12-15 days.
Test reading: Beginning of sporulation on sensitive plants approximately 12 days after the inoculation.

Test reliability:

Differential hosts to be used:

Hosts	Pathotype 1 (Verte de Cambrai)	Pathotype 2 (Gala)
Verte de Cambrai	S	R
Verella	R	S
Gala	R	S

S = Sensitive, R = Resistant

9. LITERATURE

Fascicule du CTPS - Novembre 1995: Tests de résistance aux maladies, Plantes potagères.

10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the [CPVO website](#) under the following reference:
CPVO/TQ-075/2-Rev – *Valerianella locusta* L.; *Valerianella eriocarpa* Desv. – cornsalad

Link to e-TQ:

<https://online.plantvarieties.eu/backOfficeFormQuestions?viewFormId=15480&viewFormType=TQ&viewFormLang=EN&speciesIds=VAL01&status=1,2&order=formName>