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

PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Lactuca sativa L.

LETTUCE

UPOV Code: LACTU_SAT

Adopted on 19/04/2016

Entry into force on 19/04/2016

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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 ***Lactuca sativa*** **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/en/publications/intro_dus.htm), its associated TGP documents
(http://www.upov.int/en/publications/tgp/) and the relevant UPOV Test Guideline TG/013/10 Rev.2 dated 20/03/2013 (http://www.upov.int/edocs/tgdocs/en/tg013.pdf) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **19.04.2016**. Any on-going 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 http://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette 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 independent 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/export/sites/upov/en/publications/tgp/documents/tgp_9_1.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 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/export/sites/upov/en/publications/tgp/documents/tgp_9_1.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/export/sites/upov/en/publications/tgp/documents/tgp_10_1.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

For the assessment of uniformity, 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/export/sites/upov/en/publications/tgp/documents/tgp_11_1.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.

In the first place, the collection should be divided according to the following growth types (for further information see chapter 8.1):

	Examples
1. Butterhead lettuce	Clarion, Merveille des quatre saisons, Verpia
2. Crisphead lettuce	Blonde de Paris (Batavia), Calmar, Saladin (Iceberg)
3. Cos (Roman) lettuce	Blonde maraîchere (Roman types)
4. "Grasse" or Latin lettuce	Bibb, Sucrine
5. Cutting/Gathering lettuce	Frisé d'Amérique, Lollo rossa, Oakleaf, Salad Bowl
6. Stem lettuce	Celtuce

5.3 The following have been agreed as useful grouping characteristics.

a) Seed: colour (characteristic 1)

b) Leaf: anthocyanin coloration (characteristic 18)

c) Time of beginning of bolting under long day conditions (characteristic 33)

d) Resistance to downy mildew (*Bremia lactucae*): Isolate Bl:16 (characteristic 37.7)

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.

For varieties testing resistance "present" (9) to *Bremia lactucae* Isolate Bl: 16 (characteristic 37.1), it is compulsory to test Isolates Bl: 20, 21, 26 and 27 (characteristics 37.3, 37.4, 37.9, 37.10, hence with marked [*]). For varieties testing resistance "present" (9) to *Bremia lactucae* Isolate Bl: 29 (characteristic 37.11), it is compulsory also to test Isolates Bl: 30 and Bl: 31 (characteristics 37.12 and 37.13, hence with marked [*]).

In accordance to the approval by the CPVO Administrative Council on 01/10/2015 of the procedure "The use of disease resistance characteristics in CPVO vegetable Technical Protocols" (DOC-AC-2015-2-18), a phasing-in period is established for the new asterisked diseases resistance characteristics set out in the present protocol TP-13/5 Rev. The phasing-in period for TP-13/5 Rev. has been established for three years, and will cease to apply on 01/01/2019, at which time the characteristics in question will become obligatory.

The characteristics in question are the following:

- [*] 37.10: Resistance to downy mildew (*Bremia lactucae*) Isolate Bl:27
- (*) 37.11: Resistance to downy mildew (*Bremia lactucae*) Isolate Bl:29
- [*] 37.12: Resistance to downy mildew (*Bremia lactucae*) Isolate Bl:30
- [*] 37.13: Resistance to downy mildew (*Bremia lactucae*) Isolate Bl:31

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

G	Grouping characteristic	– see Chapter 5
(*)	Asterisked characteristic	– see Chapter 6.1.2
[*]	Asterisked characteristics under certain circumstances – see Chapter 6.1.2	
MG, MS, VG, VS	– see Chapter 4.1.5	
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**	**Seed: colour**		
QL			white	Verpia	1
			yellow	Durango	2
G			black	Kagraner Sommer	3
2. (+)	**2. (*)**	**VG**	**Seedling: anthocyanin coloration**		
QL			absent	Verpia	1
			present	Pirat	9
3.	**5.**	**VG**	**Leaf: attitude at 10-12 leaf stage**		
QN			erect	Baby Star, Romance	1
			semi-erect	Great Lakes 118, Soraya	3
			prostrate	Unicum, Vanguard 75	5
4. (+)	**6.**	**VG**	**Leaf blade: division (as for 5)**		
PQ			entire	Fiorella, Sunrise	1
			lobed	A couper à feuille de chêne blonde à graine noire, Salad Bowl	2
			divided	Lagon, Monet	3
5.	**7. (*)**	**VG**	**Plant: diameter**		
QN		**(a)**	very small	Pavane, Tom Thumb	1
			small	Bastion, Gotte à graine blanche	3
			medium	Clarion, Verpia	5
			large	Great Lakes 659, Musette	7
			very large	El Toro, Yuma	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6.	**8. (*)**	**VG**	**Plant: head formation**		
PQ		**(a)**	no head	Blonde à couper améliorée, Lollo rossa	1
			open head	Manfred, Monet	2
			closed head (overlapping)	Kelvin, Sunrise	3
7.	**9.**	**VG**	**Varieties with closed head formation only: Head: degree of overlapping of upper part of leaves**		
QN		**(a)**	very weak	Colorado	1
			weak	Danilla, Novita	3
			medium	Augusta, Fiorella	5
			strong	Master, Minas	7
			very strong	Kelvin, Roxette	9
8.	**10.**	**VG**	**Head: density**		
QN		**(a)**	very loose	Ninja	1
			loose	Danilla, Nanda	3
			medium	Blonde maraîchère	5
			dense	Hilde II, Kelvin	7
			very dense	Musette, Toronto	9
9.	**11.**	**VG**	**Head: size**		
QN		**(a)**	very small	Tom Thumb	1
			small	Bastion, Gotte à graine blanche	3
			medium	Fiorella, Soraya	5
			large	Great Lakes 659, Musette	7
			very large	Blonde maraîchère, El Toro	9
10.	**12.**	**VG**	**Butterhead type varieties in closed head formation only: Head: closing of base**		
QN		**(a)**	weak	Passe Partout	3
			medium	Carmelita	5
			strong	Dustin, Manfred	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
11. (+)	**13. (*)**	**VG**	**Head: shape in longitudinal section**		
PQ		**(a)**	narrow elliptic	Verte maraîchère	1
			broad elliptic	Amadeus, Sucrine	2
			circular	Passe Partout, Verpia	3
12.	**14.**	**VG**	**Leaf: thickness**		
QN		**(a)**	thin	Raisa, Royal Red	3
			medium	Dustin, Sunrise	5
			thick	Frisée de Beauregard	7
13.	**15.**	**VG**	**Leaf: attitude at harvest maturity (outer leaves from head lettuce or adult leaves from cutting and stem lettuce)**		
QN		**(a)**	erect	Feria, Riva	1
			semi-erect	Amelia, Toronto	3
			horizontal	Chambery, Divina	5
14. (+)	**16. (*)**	**VG**	**Leaf: shape**		
PQ		**(a)**	narrow elliptic	Riva, Verte maraîchère	1
			medium elliptic	Angela, Xanadu	2
			broad elliptic	Amadeus, Amelia	3
			circular	Elsa, Sunrise, Verpia	4
			transverse broad elliptic	Commodore, Fiorella	5
			transverse narrow elliptic	Elvira, Madison	6
			obovate	Raisa, Toronto	7
			broad obtrullate	Delicato, Monet	8
			triangular	Deer Tongue	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
15.	**17.**	**VG**	**Leaf: shape of tip**		
PQ		**(a)**	acute	Celtuce, Deer Tongue, Karola, Tempra	1
			obtuse	Chicon des Charentes, Grise maraîchère	2
			rounded	Blonde Maraîchère, Maserati	3
16. (+)	**18. (*)**	**VG**	**Leaf: hue of green colour of outer leaves**		
PQ		**(a)**	absent	Donatello, Verpia	1
			yellowish	Dorée de printemps	2
			greyish	Celtuce, Du bon jardinier	3
			reddish	Lollo rossa, Revolution, Rosa (see also Ad. 18)	4
17. (+)	**19. (*)**	**VG**	**Leaf: intensity of colour of outer leaves**		
QN		**(a)**	very light	(see also Ad. 16)	1
			light	(see also Ad. 16)	3
			medium	(see also Ad. 16)	5
			dark	(see also Ad. 16)	7
			very dark	(see also Ad. 16)	9
18.	**20. (*)**	**VG**	**Leaf: anthocyanin coloration**		
QL		**(a)**	absent	Fiorella, Sunrise	1
G			present	Commodore, Pirat	9
19.	**21. (*)**	**VG**	**Leaf: intensity of anthocyanin coloration**		
QN		**(a)**	very weak	Chicon de Charentes, Muranta, Rumina	1
			weak	Du bon jardinier	3
			medium	Trocadéro à graine noire	5
			strong	Amandine, Merveille des quatre saisons	7
			very strong	Little Leprechaun, Revolution	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
20.	**22.**	**VG**	**Leaf: distribution of anthocyanin**		
QL		**(a)**	localised	Muranta, Rumina	1
			entire	Delicato, Liberty	2
21.	**23.**	**VG**	**Leaf: kind of anthocyanin distribution**		
QL		**(a)**	diffused only	Amandine, Pirat, Sanguine	1
			in spots only	Passion blonde à graine blanche, Unicum	2
			diffused and in spots	Lovina, Rougette du Midi	3
22.	**24.**	**VG**	**Leaf: glossiness of upper side**		
QN		**(a)**	absent or very weak	Divina, Du bon jardinier	1
			weak	Elsa, Fiorella	3
			medium	Feria, Sunrise	5
			strong	Ibis, Noisette	7
23.	**25. (*)**	**VG**	**Leaf: blistering**		
QN		**(a)**	absent or very weak	Donia, Frillblond	1
			weak	Fiorella, Minas	3
			medium	Commodore	5
			strong	Blonde de Paris, Smile	7
			very strong	Blonde de Doulon	9
24.	**26.**	**VG**	**Leaf: size of blisters**		
QN		**(a)**	small	Dorée de printemps	3
			medium	Dustin, Sunrise	5
			large	Fiorella, Massilia	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
25.	**27. (*)**	**VG**	**Leaf blade: degree of undulation of margin**		
QN		**(a)**	absent or very weak	Dustin, Manfred	1
			weak	Commodore, Sunrise	3
			medium	Noisette, Pentared	5
			strong	Calmar, Invicta	7
			very strong	Lollo rossa, Madison	9
26.	**28.**	**VG**	**Leaf blade: incisions of margin on apical part**		
QL		**(a)**	absent	Verpia	1
			present	Calmar, Gloire du Dauphiné, Unicum	9
27.	**29. (*)**	**VG**	**Leaf blade: depth of incisions on margin on apical part**		
QN		**(a)**	shallow	Pentared, Unicum	3
			medium	Ithaca Great Lakes	5
			deep	Lagon, Monet	7
28.	**30.**	**VG**	**Leaf blade: density of incisions on margin on apical part**		
QN		**(a)**	sparse	Maravilla de Verano	3
			medium	Calmar, De Pierre Benite	5
			dense	Grand Rapids, Ithaca Great Lakes	7
			very dense	Locarno, Madison	9
29.	**31.**	**VG**	**Varieties with shallow incisions on margin on apical part only: Leaf blade: type of incisions on apical part**		
QL		**(a)**	sinuate	Gloire du Dauphiné	1
			dentate	Calmar	2
30.	**32.**	**VG**	**Leaf blade: venation**		
QL		**(a)**	not flabellate	Donatella, Verpia, Xanadu	1
			flabellate	Gloire du Dauphiné, Locarno, Monet	2

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
31.	**33.**	**VG**	**Axillary sprouting**		
QN			absent or very weak	Valmaine	1
			weak	Aprilia, Sunrise	3
			medium		5
			strong	Riva	7
			very strong	Doncella	9
32.	**34.**	**MG**	**Time of harvest maturity**		
QN		**(a)**	very early	Blonde à couper améliorée	1
			early	Attraction	3
			medium	Newton	5
			late	Calmar	7
			very late	El Toro	9
33.	**35. (*)**	**MG**	**Time of beginning of bolting under long day conditions**		
QN			very early	Blonde à couper améliorée	1
			early	Gotte à graine blanche	3
			medium	Carelia	5
			late	Hilde II	7
G			very late	Erika, Kinemontepas, Rex	9
34.	**36.**	**VG/MG**	**Plant: height (flowering plant)**		
QN			short	Gotte à graine blanche	3
			medium	Samourai	5
			tall	Danilla, Hilde II	7
35.	**37.**	**VG**	**Plant: fasciation (at flowering stage)**		
QL			absent	Calmar, Romance	1
			present	Gotte jaune d'or	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
36.	**38.**	**VG**	**Plant: intensity of fasciation (flowering plant)**		
QN			very weak	Gotte à graine blanche	1
			weak	Verte maraîchère	3
			medium	Amadeus	5
			strong	Gotte jaune d'or	7
			very strong	Chicon des Charentes	9
37. (+)	**39.**	**VG**	**Resistance to downy mildew (*Bremia lactucae*)**		
37.1 (*)	**39.7 (*)**		**Isolate Bl: 16**		
QL		**(b) (c)**	absent	Green Towers	1
G			present	Argelès, Ninja	9
37.2	**39.8**		**Isolate Bl: 17**		
QL		**(b) (c)**	absent	Green Towers	1
			present	Argelès, Ninja	9
37.3 (*)	**39.10**		**Isolate Bl: 20**		
QL		**(b) (c)**	absent	Green Towers	1
			present	Argelès, Ninja	9
37.4 (*)	**39.11**		**Isolate Bl: 21**		
QL		**(b) (c)**	absent	Green Towers	1
			present	Argelès, Colorado	9
37.5	**39.12**		**Isolate Bl: 22**		
QL		**(b) (c)**	absent	Green Towers	1
			present	Discovery, Ninja	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
37.6	**39.13**		**Isolate Bl: 23**		
QL		**(b) (c)**	absent	Green Towers	1
			present	Colorado, Discovery, Ninja	9
37.7	**39.14**		**Isolate Bl: 24**		
QL		**(b) (c)**	absent	Argelès, Colorado	1
			present	Dandie, NunDm15, UC DM14	9
37.8	**39.15**		**Isolate Bl: 25**		
QL		**(b) (c)**	absent	Colorado, Discovery	1
			present	Argelès, Ninja	9
37.9 (*)	**39.16**		**Isolate Bl: 26**		
QL		**(b) (c)**	absent	Colorado, Discovery	1
			present	Balesta, Bedford	9
37.10 (*)	**39.17**		**Isolate Bl: 27**		
QL		**(b) (c)**	absent	Balesta, Colorado	1
			present	Bedford, Discovery	9
37.11 (*)			**Isolate Bl: 29**		
QL		**(b) (c)**	absent	Argelès, Discovery	1
			present	Balesta, Ninja	9
37.12 (*)			**Isolate Bl: 30**		
QL		**(b) (c)**	absent	Argelès, Colorado	1
			present	Balesta, Ninja	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
37.13 (*)			**Isolate Bl: 31**		
QL		**(b) (c)**	absent	Colorado, RYZ910457	1
			present	Argelès, Balesta	9
38. (+)	**40.**	**VG**	**Resistance to lettuce mosaic virus (LMV)**		
QL		**(b) (c)**	**Stain Ls 1**		
			absent	Hilde II, Salvina	1
			present	Corsica	9
39. (+)		**VG**	**Resistance to *Nasonovia ribisnigri* biotype N°: 0**		
			absent	Green Towers, Abel, Nadine	1
			present	Silvinas, Barcelona, Dynamite	9

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 Key to Lettuce Types (under Section 5.3)

Cultivated lettuce varieties (vegetables) can be grouped into the following growth types:

(1) Butterhead Lettuce

Heading or with a tightly filled heart, thin to medium thick tender leaves with a clear midrib; head shape ranging from broad elliptic to transverse elliptic.

(2) Crisphead Lettuce (including the Iceberg, Batavia and Maravilla types)

Weak to very strong heading, rather thin to very thick and tough leaves, no clear midrib but with flabellate venation. Iceberg types (like Calmar and Saladin) are mainly thick and tough-leaved, predominantly green and greygreen, leaf margin hardly to rather strongly incised.
Batavia types are generally medium thick-leaved and with rather strongly blistered leaves, predominantly yellowish or medium green; under cold conditions not always clearly heading.
Maravilla types have rather thick and tough leaves, only slightly or not blistered.

(3) Cos Lettuce (Roman Lettuce)

Heading or semi-heading, elongated and rather tough leaves with a clear midrib, head shape in longitudinal section elliptic, length of head >1.5 x diameter.

(4) "Grasse" or Latin Lettuce (sometimes included under Cos Lettuce)

Heading or semi-heading, tough thick leaves with clear midrib, head shape short elliptic to slightly obovate. Some types only have a tightly filled heart, others are more similar to a short Cos Lettuce. Suitable for semi-arid conditions.

(5) Cutting or Gathering Lettuce

Rather heterogeneous group ranging from non-heading butterhead-like, non-heading Batavia-like, non-heading crisp types to Oakleaf and Catalogna (lobed) types with deeply dissected leaves (Monet) and types with strongly undulated leaf margin (Lollo). Varieties partly with a clear midrib and partly with flabellate venation of the leaves. Common characteristic: loose-leaved rosette.

(6) Stem Lettuce

Forms a fleshy stem before bolting, at least under (semi-) short day conditions; leaves are mainly tough and have a clear midrib. Leaves and/or stem are consumed

8.2 Explanations covering several characteristics

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

(a) Plant, head, leaf, leaf blade: Observations on the plant, head, leaf and leaf blade should be made at harvest maturity.

(b) Disease resistance: When disease resistance characteristics are used for assessing distinctness, uniformity and stability, records should be taken under conditions of controlled infection with a defined pathotypes.

(c) Resistance to downy mildew: Each race should be tested separately and the results should also be indicated separately (b) etc.

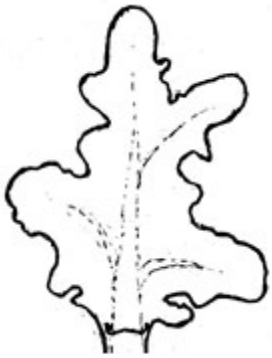
8.2 Explanations for individual characteristics

Ad. 2: Seedling: anthocyanin coloration

This characteristic can easily be observed by keeping the remaining seedlings after pricking out in the seeding tray without watering and under cold(er) conditions. Within two or three days all seedlings of varieties with anthocyanin will show this characteristic.

Ad. 4: Leaf blade: division

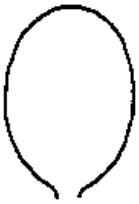
1
entire



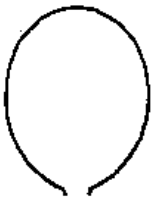
2
lobed

3
divided

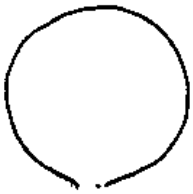
Ad. 11: Head: shape in longitudinal section



1
narrow elliptic



2
broad elliptic



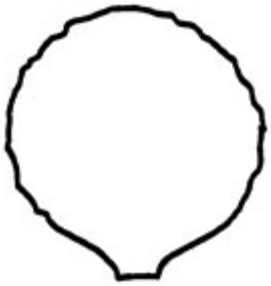
3
circular

Ad. 14: Leaf: shape

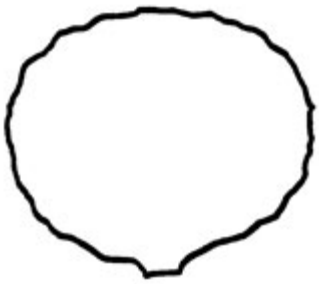
1
narrow elliptic

2
medium elliptic

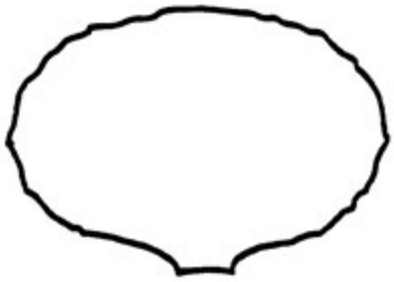
3
broad elliptic



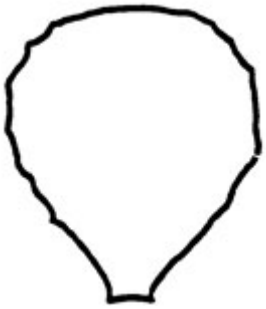
4
circular



5
transverse broad elliptic



6
transverse narrow elliptic



7
obovate

8
broad obtrullate

9
triangular

Ad. 16: Leaf: hue of green colour of outer leaves
Ad. 17: Head: intensity of colour of outer leaves

Intensity of colour (Ch. 19)	Hue of green colour (Ch. 18)			
	1 absent	2 yellowish	3 greyish	4 reddish
1 very light	Krizet	Marbello Black Seeded Simpson	Hohlblättriger Butter	
3 light	Blonde maraîchère, Mondial, Reskia	Blondine (= Viktoria), Locarno, Pia	Celtuce, Kinemontepas, Natina	Brauner Trotzkopf, Maravilla de Verano
5 medium	Florian, Frillblond, Sunrise, Têtue de Nîmes	Australische Gele, Dorée de printemps, Gotte jaune d'or	Clarion, Du bon jardinier, Durango, Kelvin	Lollo rossa, Pirat, Prizehead (= Frisée d'Amérique)
7 dark	Baby Star, Donatello, Verpia, Waldemann Dark Green	Batavia, Chicon	Chou de Naples (= Webb's Wonderful), Galaxy, Toledo	Merveille des quatre saisons, Rosa, Rouge d'Hiver
9 very dark	Pavane		(Sudia)	Liberty, Malibu, Pentared, Revolution

Ad. 37: Resistance to downy mildew (*Bremia lactucae*)

1. Pathogen ..... *Bremia lactucae*
2. Quarantine status ..... -
3. Host species ..... *Lactuca sativa* L.
4. Source of inoculum ..... GEVES (FR) or Naktuinbouw (NL)
5. Isolate ..... Bl: 1 – Bl: 27 (see table below)
6. Establishment isolate identity ..... test on differentials
7. Establishment pathogenicity ..... test on susceptible varieties
8. Multiplication inoculum
8.1 Multiplication medium ..... lettuce leaf
8.2 Multiplication variety ..... susceptible variety, for example Green Towers for higher races, a variety with defeated resistance may be preferable to keep the isolate fit
8.3 Plant stage at inoculation ..... cotyledon to first leaf
8.4 Inoculation medium ..... tap water
8.5 Inoculation method ..... spraying a spore suspension
8.6 Harvest of inoculum ..... washing off from leaves
8.7 Check of harvested inoculum ..... counting spores
8.8 Shelf life/viability inoculum ..... 2 hours at room temperature; 2 days in fridge
9. Format of the test
9.1 Number of plants per genotype ..... normally 60, minimum 20
9.2 Number of replicates ..... 1 replicate
9.3 Control varieties ..... (informative) differentials
9.3 Control varieties ..... (informative) differentials
9.4 Test design ..... include control varieties
9.5 Test facility ..... climate room
9.6 Temperature ..... 15°C-17°C
9.7 Light ..... adequate for good plant growth;
seedlings should not etiolate;
reduced light 24 hours after inoculation

9.9 Special measures	Plants may grow on wet blotting paper with or without a nutrient solution, or on potting soil. High humidity (>90%) is essential for infection and sporulation.
10. Inoculation	
10.1 Preparation inoculum.....	washing off from leaves by vigorous shaking in a closed container
10.2 Quantification inoculum	counting spores ; spore density should be 3.10^4-1.10^5
10.3 Plant stage at inoculation.....	cotyledon stage
10.4 Inoculation method	spraying till run-off reduced light 24 hours after inoculation
10.5 First observation	7 days after inoculation
10.6 Second observation	10 days after inoculation
10.7 Final observations	13 days after inoculation; two of these three time points may be sufficient. The day of maximum sporulation should occur in this period.
11. Observations	
11.1 Method.....	visual observation of sporulation and necrotic reaction to infection
11.2 Observation scale.....	+ 1. abundant sporulation on both sides of the cotyledon (+) 2. normal sporulation on the lower side of the cotyledon (+) 3. normal sporulation on the lower side of the cotyledons combined with necrotic spots (-) 4. sparse sporulation on the lower side of the cotyledons combined with necrosis (-) 5. necrotic pinpoints - 6. no symptoms
11.3 Validation of test.....	on standards
11.4 Off-types.....	susceptible plants in a resistant variety resistant plants in a susceptible variety 3 or less off-types in 60 plants
12. Interpretation of data.....	class 1, 2 and 3: susceptible class 4, 5 and 6: resistant
13. Critical control points:.....	Reaction of standards. The infection pressure may vary between experiments, leading to slight differences in sporulation intensity. When the reactions are not clear the experiment should be repeated.

For reference: The international Bremia evaluation board (IBEB) produces regular updates of the host differential reaction table. The most recent table is available through ISF at www.worldseed.org. The table for Bl: 16-31 is given below.

Isolates / Differentials	GreenTowers	Dandie	R4T57D	UC Dm14	NunDm15	CGDm16	Colorado	FrRsal-1	Argeles	RYZ 2164	RYZ910457	Bedford	Balesta	Bartoli	Design	Kibrille
BI: 16	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
BI: 17	+	+	-	+	+	-	+	+	-	-	-	(+)	-	-	-	-
BI: 20	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-
BI: 21	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-
BI: 22	+	-	+	+	+	-	+	-	-	-	-	-	+	-	-	-
BI: 23	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-
BI: 24	+	-	+	-	-	+	+	-	+	-	-	-	-	-	(-)	-
BI: 25	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-
BI: 26	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-
BI: 27	+	+	+	+	+	-	+	-	+	+	-	(-)	+	-	-	-
BI: 29	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
BI: 30	+	-	+	+	+	-	+	-	+	+	-	-	-	-	+	-
BI: 31	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-

Availability of *Bremia* isolates and differentials

The GEVES in France and Naktuinbouw in the Netherlands verify and test *Bremia* isolates as defined and denominated by the International *Bremia* Evaluation Board (IBEB). GEVES and Naktuinbouw are responsible for delivery of denominated isolates to the testing centres of other UPOV members against payment of prescribed fees.

The addresses of the centres are as follows:

GEVES
25 Rue Georges Morel
CS 90024
49071 Beaucouzé Cedex
France
Tél.: +33 (0) 2 41 22 58 00
Tlcp.: +33 (0) 2 41 22 58 01
Mél.: service.clients@geves.fr

Naktuinbouw
Sotaweg 22
P.O. Box 40
2370 AA Roelofarendsveen
Netherlands
Tel.: + 31 (0) 71 332 62 62
Fax.: + 31 (0) 71 332 63 63
Email: info@naktuinbouw.nl

The common differential set of lettuce varieties and lines for determination of *Bremia* isolates is available from Naktuinbouw in the Netherlands (address as above) and GEVES in France at the following address:

GEVES Brion
Domaine de la Boisselière
49250 Brion
France

Ad. 38: Resistance to Lettuce Mosaic Virus (LMV)

Maintenance of strains

Maintenance: After 15-20 days of incubation infected tissue should be sliced and desiccated over calcium chloride and stored at 4ºC. Infectivity may last 1 to 3 years. Contamination can be avoided in this way.

Multiplication: Pre-multiplication of the virus on a susceptible variety (e.g. Hilde or Trocadero) prior to testing under normal conditions. Only virus-free seed samples should be used for this purpose.

Execution of test

Growth stage of plants: First inoculation at 2 to 3 leaves stage.

Temperature: Constant temperature of 16ºC during night (N) and of 22ºC during day (D) or, alternatively, temperature of 20ºC N, 25ºC D during 5 days after inoculation followed by 12ºC N and 18ºC D.

Light conditions: From emergence: 16 hours per day, at least 15,000 Lux.

Preparation of inoculum: Young leaves of diseased lettuce plants showing clear LMV symptoms (after 15-25 days of incubation) should be ground (1 g fresh leaves per 4 ml) in a mortar adding a 0.03 M Na_2HPO_4-buffer containing 0.2% DIECA(*). Prior to inoculation 75 mg/ml carborundum and 75 mg/ml activated charcoal should be added.

(*) Composition of buffer: per 100 ml: 1.07 g Na_2HPO_4 $12H_2O$, 0.2 g DIECA

Method of inoculation: Mechanical inoculation by rubbing on the two first leaves, followed by a second inoculation 2-3 days afterwards. The inoculum is kept in an ice bucket during inoculation.

Duration of test:
- From sowing to inoculation: about 2 weeks
- rom inoculation to reading: about 2 to 3 weeks; first reading after 15 days

Number of plants tested: 30 plants and 6 repetitions

Remarks:

Strains: Other strains of LMV have been isolated in Europe (France, Greece, Spain) by Dinant and Lot (1992), Plant Pathology 41:528-542. The naming of the strains is not yet internationally accepted; but names of pathotypes have been proposed (Pink, Lot and Johnson (1992), Euphytica 63:169-174).

Symptoms (under test conditions): The expression of the symptoms depends on the strains and the lettuce genotypes. For the old Ls-1 strain used for testing the 'Gallega'-gene, the typical reactions can be summarized as follows:

· Butterhead cultivars show essentially vein clearing and mosaic;

· Crisp or Iceberg cultivars show chlorosis along the veins and faint mosaic;

· Cos cultivars show reduced growth of the inner leaves and blistering;

· In red varieties symptoms are particularly difficult to observe.

Ad. 39: Resistance to *Nasonovia ribisnigri* biotype N°: 0

Maintenance of biotype

Nasonovia ribisnigri is a leaf aphid and may be maintained alive on susceptible lettuce plants in aphid-proof chambers or tents in a glasshouse. *N. ribisnigri* is usually green, but some biotypes are red. A red aphid is easier to see on a green leaf. Therefore red biotypes are usually preferable. The aphid's body size is 1.5-2.5 mm. The body has 7 dark spots. The ends of the legs are black.

The common biotype N°: 0 can be distinguished from resistance-breaking biotypes by means of a biotest using a suitable resistant control variety, for example Silvinas.

Multiplication:
On a susceptible variety at 20-22°C for 10-14 days. Aphids are shaken off into a Petri-dish.

Sowing:
12°C for germination and early growth; plant distance at least 5 cm.
Number of plants to be tested: 28.

Inoculation method:
Careful transfer of 5 aphids per plant using a fine paintbrush.
Plant stage at inoculation: 15 days.
Temperature: 20-22°C.

Observation:
First observation: 10 days after inoculation.
Second observation: daily check whether newborn aphids are mature (= red).
End of test: max. 15 days after inoculation.

Observation at end of test: Count the number of mature (= red) aphids on each plant.

Scale for observations:		Interpretation of data
0	no aphids	Resistant
1	1-5 aphids per plant	Resistant
2	6-10 aphids per plant	Undecided
3	> 10 aphids per plant	Susceptible

Remarks
Resistant control varieties and susceptible control varieties should have at least 95% (26/28) resistant plants and susceptible plants, respectively.
If more than 2 of 28 plants of the control varieties are undecided or off type, the experiment should be repeated.

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference: CPVO-TQ/013/5 Rev.