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

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CPVO-TP/013/6-Rev.5 based on the version 5 of the CPVO-TP/template

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CPVO-TP/013/6-Rev.5

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/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/13/11 Rev. 2 dated 26/10/2021 (https://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 **01.01.2025**. 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/she 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/tgpdocs/en/tgp_9.pdf.

3.3 Conditions for Conducting the Examination

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

3.4 Test design

3.4.1 Each test should be designed to result in a total of at least 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 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.

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.

For testing the resistance to certain pathogens, unless otherwise indicated, the test should be performed on at least 20 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.

4.2.3 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

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.pd)

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) Seed: colour (characteristic 1)

b) Leaf: anthocyanin coloration (characteristic 11)

c) Time of beginning of bolting (characteristic 35)

d) Resistance to *Bremia lactucae* (Bl) isolate Bl: 29EU (characteristic 38)

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".

Table 1

Type	**Example varieties**	**Plant: degree of overlapping of upper part of leaves (Char. 3)**	**Leaf: number of divisions (Char. 6)**	**Leaf: thickness (Char. 17)**	**Leaf: undulation of margin (Char. 20)**	**Leaf: venation (Char. 25)**	**Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Head: shape in longitudinal section (Char. 27)**
Butterhead type	Clarion, Maikönig, Sartre	medium to strong	absent or very few	thin to thick	absent to weak	not flabellate	circular or narrow oblate
Novita type	Norvick	absent or weak	absent or very few	thin to medium	very weak to medium	flabellate	-
Iceberg type	Great Lakes 659, , Saladin, Toscanas, Vanguard 75	strong	absent or very few	thick	absent to medium	flabellate	circular or narrow oblate
Batavia type	Aquarel, Curtis, Decision, Funnice, Felucca, Grand Rapids, Masaida	absent or weak to strong	absent or very few	medium to thick	weak to very strong	flabellate	broad elliptic, circular or narrow oblate
Frisée d'Amérique type	Bijou, Blonde à couper améliorée	absent or weak	absent or very few	thin	absent to strong	flabellate or not flabellate or semi	-
Lollo type	Lollo rossa, Revolution	absent or weak	absent or very few	thin	strong to very strong	flabellate	-
Oakleaf type	Catalogna, Kipling, Muraï, Salad Bowl	absent or weak	few to many	thin	absent to weak	flabellate or not flabellate or semi	-
Multi-divided type	Curletta, Duplex, Jadigon, Rodagio	absent or weak	medium to very many	thin	weak to very strong	flabellate	-
Frillice type	Frilett	absent or weak	absent or very few	thick	weak to strong	flabellate	-
Cos type	Actarus, Blonde maraîchère, Pinokkio	absent or weak to medium	absent or very few	medium to thick	absent to weak	not flabellate	narrow elliptic
Gem type	Craquerelle du Midi, Sucrine, Xanadu	absent or weak to medium	absent or very few	medium to thick	absent to weak	not flabellate	broad elliptic, circular or narrow oblate
Stem type	Celtuce, Guassihong	absent or weak	absent or very few	thin to medium	absent to weak	not flabellate	-

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.

Phasing in period

The following new asterisked disease resistances:
- characteristics 44: Resistance to *Bremia lactucae* isolate Bl: 38EU
- characteristics 45: Resistance to *Bremia lactucae* isolate Bl: 39EU
- characteristics 46: Resistance to *Bremia lactucae* isolate Bl: 40EU

have been introduced in CPVO/TP-013/6-Rev.4. The phasing in period **[*]** for those new characteristics have been established for three years, and will cease to apply on 01/01/2027, at which time the characteristics in question for CPVO/TP-013/6-Rev.4 will become obligatory.

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
[*]	New asterisked disease resistance characteristic subject to the phasing-in period	-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.

For column 'Stage, method':

MG, MS, VG, VS		-see Chapter 4.1.5
(a)-(b)	Explanations covering several Characteristics	-see Chapter 8.1
Lettuce types		-see Chapter 8.3

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1.	**1. (*)**	**VG**	**Seed: colour**		
PQ			white	Verpia	1
			yellow	Durango	2
			brown	Oaklin	3
G			black	Kagraner Sommer 2	4
2.	**2. (*)**	**MS/VG**	**Plant: diameter**		
QN		**(a)**	very small	Tom Thumb	1
			very small to small		2
			small	Gotte à graine blanche	3
			small to medium		4
			medium	Clarion, Verpia	5
			medium to large		6
			large	Great Lakes 659	7
			large to very large		8
			very large	El Toro	9
3. (+)	**3. (*)**	**VG**	**Plant: degree of overlapping of upper part of leaves**		
QN		**(a)**	absent or weak	Actarus, Aquarel, Blonde à couper améliorée, Curtis, Lollo rossa	1
			medium	Augusta, Clarion, Fiorella	2
			strong	Toscanas, Vanguard 75	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
4. (+)	**4.**	**MS/VG**	**<u>Only varieties with Plant: degree of overlapping of upper part of leaves: absent or weak:</u> Plant: number of leaves**		
QN		**(a)**	very few		1
			very few to few		2
			few	Lollo rossa	3
			few to medium		4
			medium	Muraï	5
			medium to many		6
			many	Felucca, Sartre, Xandra	7
			many to very many		8
			very many		9
5. (+)	**5.**	**VG**	**Leaf: attitude**		
QN		**(b)**	erect	Feria, Pinokkio	1
			erect to semi-erect		2
			semi-erect	Expedition, Sartre	3
			semi-erect to horizontal		4
			horizontal	Divina	5
6. (+)	**6. (*)**	**VG**	**Leaf: number of divisions**		
QN		**(b)**	absent or very few	Fiorella, Lollo rossa	1
			very few to few		2
			few	Curletta, Rodagio	3
			few to medium		4
			medium	Ezabel, Jadigon	5
			medium to many		6
			many	Expedition, Multired 54	7
			many to very many		8
			very many	Excite, Ezfrill, Telex	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
7. (+)	**7.**	**VG**	**<u>Only varieties with Leaf: number of divisions: absent or very few:</u> Leaf: shape**		
PQ		**(b)**	triangular		1
			lanceolate	Qingyuanyewoju	2
			medium oblate	Stylist	3
			narrow oblate	Commodore, Fiorella	4
			circular	Verpia	5
			broad elliptic	Amadeus	6
			medium elliptic	Xanadu	7
			narrow elliptic	Verte maraîchère	8
			linear	Hongwoju	9
			broad obtrullate		10
			obovate	Aquino	11
			oblanceolate	Xiangshengcai	12
8. (+)	**8.**	**VG**	**<u>Only varieties with Leaf: number of divisions: absent or very few:</u> Leaf: shape of apex**		
PQ		**(b)**	acute	Celtuce	1
			obtuse	Actarus	2
			rounded	Blonde maraîchère, Maserati	3
			obcordate	PS 6545691	4
9. (+)	**9.**	**VG**	**<u>Only varieties with Leaf: number of divisions: absent or very few:</u> Leaf: longitudinal section**		
QN		**(b)**	concave	Sunstar	1
			concave to flat		2
			flat	Clarion, Lollo rossa	3
			flat to convex		4
			convex	Tiago	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
10. (+)	**10.**	**VG**	**<u>Only Oakleaf type varieties:</u> Leaf: width of lobes**		
QN		**(b)**	very narrow		1
			very narrow to narrow		2
			narrow	Kibrille, Rougini	3
			narrow to medium		4
			medium	Bandolin, Ribaï	5
			medium to broad		6
			broad	Horix, Starix, Vizir	7
			broad to very broad		8
			very broad		9
11. (+)	**11. (*)**	**VG**	**Leaf: anthocyanin coloration**		
QN		**(b)**	absent or very weak	Clarion	1
			very weak to weak		2
			weak	Du bon jardinier	3
			weak to medium		4
			medium	Lollo rossa, Luana	5
			medium to strong		6
			strong	Merveille des quatre saisons	7
			strong to very strong		8
G			very strong	Iride, Revolution	9
12.	**12. (*)**	**VG**	**Leaf: hue of anthocyanin coloration**		
PQ		**(b)**	reddish	Lollo rossa	1
			purplish	Iride	2
			brownish	Luana, Maravilla de Verano	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
13. (+)	**13.**	**VG**	**Leaf: area covered by anthocyanin coloration**		
QN		**(b)**	very small	Steirer Krauthauptel	1
			very small to small		2
			small	Diablo	3
			small to medium		4
			medium	Luana	5
			medium to large		6
			large	Merveille des quatre saisons	7
			large to very large		8
			very large	Bijou, Revolution	9
14. (+)	**14. (*)**	**VG**	**Leaf: colour**		
PQ		**(b)**	green	Verpia	1
			yellowish green	Dorée de printemps	2
			greyish green	Celtuce, Du bon jardinier	3
15.	**15. (*)**	**VG**	**Leaf: intensity of green colour**		
QN		**(b)**	very light		1
			very light to light		2
			light	Blonde maraîchère, Lollo Bionda	3
			light to medium		4
			medium	Aquarel, Clarion	5
			medium to dark		6
			dark	Expedition, Verpia	7
			dark to very dark		8
			very dark	Pascal, Verdetrix	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
16.	**16.**	**VG**	**Leaf: glossiness of upper side**		
QN		**(b)**	absent or very weak	Divina, Du bon jardinier	1
			very weak to weak		2
			weak	Duplex, Fiorella, Sartre	3
			weak to medium		4
			medium	Funnice	5
			medium to strong		6
			strong	Noisette, Redair	7
			strong to very strong		8
			very strong	Bijou	9
17.	**17. (*)**	**VG**	**Leaf: thickness**		
QN		**(b)**	very thin	Stefano	1
			thin	Bijou, Lollo rossa	2
			medium	Curtis, Expedition	3
			thick	Frilett, Toscanas	4
			very thick	PS 6545691	5
18.	**18. (*)**	**VG**	**Leaf: blistering**		
QN		**(b)**	absent or very weak	Duplex, Sartre	1
			very weak to weak		2
			weak	Fiorella	3
			weak to medium		4
			medium	Commodore	5
			medium to strong		6
			strong	Blonde de Paris, Xanadu	7
			strong to very strong		8
			very strong	Blonde de Doulon, Iride, Karioka	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
19. (+)	**19.**	**VG/VS**	**Leaf: size of blisters**		
QN		**(b)**	very small		1
			very small to small		2
			small	Dorée de printemps, Rodagio	3
			small to medium		4
			medium	Clarion	5
			medium to large		6
			large	Fiorella	7
			large to very large		8
			very large		9
20. (+)	**20. (*)**	**VG/VS**	**Leaf: undulation of margin**		
QN		**(b)**	absent or very weak	Tiago	1
			very weak to weak		2
			weak	Commodore	3
			weak to medium		4
			medium	Noisette, Pentared	5
			medium to strong		6
			strong	Calmar, Invicta	7
			strong to very strong		8
			very strong	Lollo rossa	9
21. (+)	**21.**	**VG**	**Leaf: type of incisions of margin**		
PQ		**(b)**	crenate	Gloire du Dauphiné	1
			regularly dentate	Soliflore	2
			irregularly dentate	Rodagio	3
			bidentate	Great Lakes 118	4
			tridentate	Expedition	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
22. (+)	**22.**	**VG**	**Leaf: depth of incisions of margin**		
QN		**(b)**	absent or very shallow	Actarus, Clarion, Tiago	1
			very shallow to shallow		2
			shallow	Pentared, Unicum	3
			shallow to medium		4
			medium	Santarinas	5
			medium to deep		6
			deep	Expedition	7
			deep to very deep		8
			very deep		9
23. (+)	**23.**	**VG**	**Only varieties with Leaf: type of incisions of margin: irregularly dentate, bi- or tridentate: Leaf: depth of secondary incisions of margin**		
QN		**(b)**	very shallow		1
			very shallow to shallow		2
			shallow	Great Lakes 659	3
			shallow to medium		4
			medium	Expedition	5
			medium to deep		6
			deep		7
			deep to very deep		8
			very deep		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
24. (+)	**24.**	**VG**	**Leaf: density of incisions of margin**		
QN		**(b)**	very sparse		1
			very sparse to sparse		2
			sparse	Maravilla de Verano	3
			sparse to medium		4
			medium	Calmar	5
			medium to dense		6
			dense	Grand Rapids	7
			dense to very dense		8
			very dense	Locarno	9
25. (+)	**25. (*)**	**VG**	**Leaf: venation**		
QN		**(b)**	not flabellate	Verpia, Xanadu	1
			semi-flabellate	Kibrille, Muraï	2
			flabellate	Locarno, Toscanas	3
26.	**26.**	**MS/VG**	**Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Head: size**		
QN		**(a)**	very small	Tom Thumb	1
			very small to small		2
			small	Xanadu	3
			small to medium		4
			medium	Fiorella, Vermekia	5
			medium to large		6
			large	Great Lakes 659	7
			large to very large		8
			very large	Blonde maraîchère, El Toro	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
27. (+)	**27. (*)**	**MS/VG**	**Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Head: shape in longitudinal section**		
QN		**(a)**	narrow elliptic	Verte maraîchère	1
			broad elliptic	Amadeus, Sucrine	2
			circular	Verpia	3
			narrow oblate	Ametist	4
28.	**28.**	**VG**	**Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Head: density**		
QN		**(a)**	very loose		1
			very loose to loose		2
			loose	Nanda	3
			loose to medium		4
			medium	Daguan, Delice	5
			medium to dense		6
			dense	Atella, Islandia	7
			dense to very dense		8
			very dense	Rubette	9
29.	**29.**	**MS/VG**	**Only Stem type varieties: Stem: length**		
(+)		**(a)**	very short		1
QN			very short to short		2
			short	Wuweijanye	3
			short to medium		4
			medium	Zipixiang	5
			medium to long		6
			long	Guasihong	7
			long to very long		8
			very long		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
30.	**30.**	**MS/VG**	**Only Stem type varieties: Stem: width**		
(+)		**(a)**	narrow	Ailaowoju	1
QN			medium	Zipixiang	2
			broad	Guasihong	3
31.	**31.**	**VG**	**Only Stem type varieties: Stem: shape in longitudinal section**		
(+)		**(a)**	cylindrical	Chiwoju	1
PQ			conical	Guasihong	2
			fusiform	Zipixiang	3
32.	**32.**	**VG**	**Only Stem type varieties: Stem: colour**		
PQ		**(a)**	whitish green	Wuweijanye	1
			light green	Chiwoju	2
			medium green	Yangwoju	3
			greenish purple	Guasihong	4
			purplish red	Hongwosun	5
33.	**33.**	**VG**	**Only Stem type varieties: Stem: colour of flesh**		
PQ		**(a)**	yellowish white	Wuweijanye	1
			whitish green	Chiwoju	2
			light green	Yangwoju	3
			medium green	Guasihong	4
			dark green	Chiwosun	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
34.	**34.**	**MG/VG**	**<u>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong</u>: Time of harvest maturity**		
QN			very early	Gotte jaune d'or	1
			very early to early		2
			early	Pantlika, Sucrine	3
			early to medium		4
			medium	Clarion	5
			medium to late		6
			late	Blonde maraîchère, Calmar	7
			late to very late		8
			very late	El Toro, Pinokkio	9
35. (+)	**35. (*)**	**MG/VG**	**Time of beginning of bolting**		
QN			very early	Blonde à couper améliorée	1
			very early to early		2
			early	Gotte à graine blanche	3
			early to medium		4
			medium	Pantlika	5
			medium to late		6
			late	Hilde II	7
			late to very late		8
G			very late	Erika, Toscanas	9
36. (+)	**36.**	**VG**	**Axillary sprouting**		
QN			absent or weak	Claridia, Shotter, Valmaine, Xanadu	1
			medium	Actarus	2
			strong	Amible, Bassoon	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
37. (+)	**37.**	**VG**	**Bolting stem: fasciation**		
QN			absent or very weak	Aquarel, Gotte à graine blanche	1
			very weak to weak		2
			weak	Verte maraîchère	3
			weak to medium		4
			medium	Amadeus	5
			medium to strong		6
			strong	Rougini	7
			strong to very strong		8
			very strong	Sartre, Verdetrix	9
38. (+) (*)	**38.**	**VS/VG**	**Resistance to *Bremia lactucae* (Bl) isolate Bl: 29EU**		
QL			absent	Argelès	1
G			present	Balesta	9
39. (+)	**39.**	**VS/VG**	**Resistance to *Bremia lactucae* (Bl) isolate Bl: 30EU**		
QL			absent	Argelès, Colorado	1
			present	Balesta	9
40. (+)	**40.**	**VS/VG**	**Resistance to *Bremia lactucae* (Bl) isolate Bl: 31EU**		
QL			absent	Colorado, RYZ910457	1
			present	Argelès, Balesta	9
41. (+)	**41.**	**VS/VG**	**Resistance to *Bremia lactucae* (Bl) isolate Bl: 33EU**		
			absent	Kibrille, RYZ2164	1
QL			present	RYZ910457	9
42. (+)	**42.**	**VS/VG**	**Resistance to *Bremia lactucae* (Bl) isolate Bl: 35EU**		
			absent	Design, Kibrille	1
QL			present	Bartoli	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
43. (+)	**43.**	**VS/VG**	**Resistance to *Bremia lactucae* (Bl) isolate Bl: 36EU**		
			absent	Bartoli, RYZ2164	1
QL			present	Design, Kibrille	9
44. (+)		**VS/VG**	**Resistance to *Bremia lactucae* (Bl) isolate Bl: 38EU**		
[*]			absent	Design, Kibrille	1
QL			present	Bartoli	9
45. (+)		**VS/VG**	**Resistance to *Bremia lactucae* (Bl) isolate Bl: 39EU**		
[*]			absent	Bartoli, RYZ2164, Dandie	1
QL			present	Design, Kibrille	9
46. (+)		**VS/VG**	**Resistance to *Bremia lactucae* (Bl) isolate Bl: 40EU**		
[*]			absent	Bartoli, RYZ2164	1
QL			present	Kibrille	9
47. (+)	**44.**	**VS/VG /MS**	**Resistance to *Lettuce mosaic virus* (LMV) pathotype II**		
QL			absent	Bijou, Hilde II, Sprinter, Sucrine	1
			present	Capitan, Corsica	9
48. (+)	**45.**	**VS/VG**	**Resistance to *Nasonovia ribisnigri* (Nr) biotype Nr: 0**		
QL			absent	Abel, Green Towers, Nadine	1
			present	Barcelona, Bedford, Dynamite, Silvinas	9
49. (+)	**46.**	**VS/VG**	**Resistance to *Fusarium oxysporum* f.sp. *lactucae* (Fol) race 1**		
QN			absent or low	Cobham Green, Patriot	1
			medium	Affic, Fuzila, Natexis	2
			high	Costa Rica No. 4, Romasol	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
50. (+)	**47.**	**VS/VG**	**Resistance to *Fusarium oxysporum* f.sp. *lactucae* (Fol) Race 4**		
			absent or low	Costa Rica No. 4, Gisela	1
QN			medium	Ballerina, Patriot	2
			high	Lomeria, Palmos	3

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 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 and stem: observations should be made at harvest maturity. For varieties with a degree of overlapping of upper part of leaves absent or weak observations should be made just before deterioration and before bolting.

b) Leaf: for varieties with a degree of overlapping of upper part of leaves medium or strong observations should be made on the largest outer leaves, at harvest maturity. For varieties with degree of overlapping of upper part of leaves absent or weak observations should be made on the largest leaves, just before deterioration and before bolting. For Stem type varieties, observations should be made on leaves at the middle third of the stem, just before deterioration and before bolting.

8.2 Explanations for individual characteristics

Ad. 3: Plant: degree of overlapping of upper part of leaves

Observations should be made on leaves at the heart of the plant to form a head.

1
absent or weak

2
medium

3
strong

Ad. 4: Only varieties with Plant: degree of overlapping of upper part of leaves: absent or weak: Plant: number of leaves

In case of doubt, observations can be made by cutting the plant in half.

3
few

5
medium

7
many

Ad. 5: Leaf: attitude

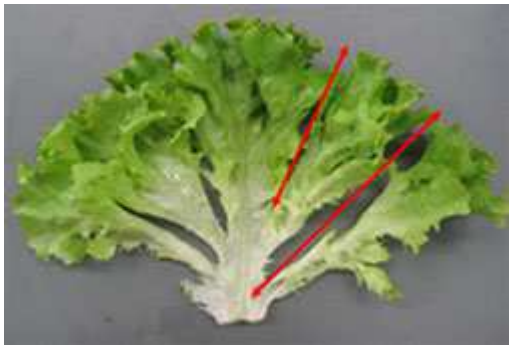
1
erect

3
semi-erect

5
horizontal

Ad. 6: Leaf: number of divisions

Observations should be made only on the incisions more than halfway to the midrib of the whole leaf.



1
absent or very few

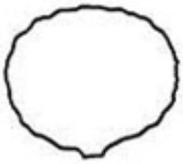
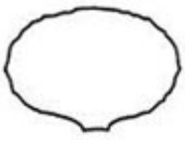
3
few

5
medium

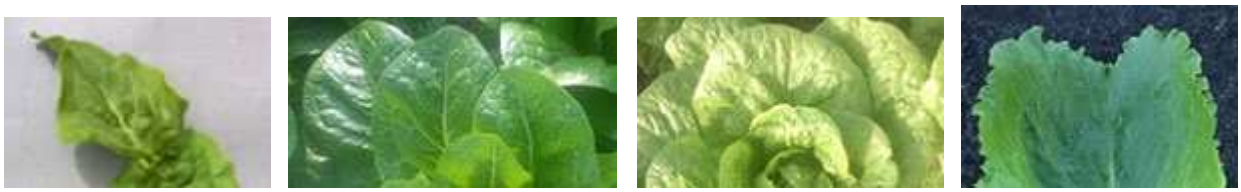
7
many

9
very many

Ad. 7: Only varieties with Leaf: number of divisions: absent or very few: Leaf: shape

	< broadest part >		
width (ratio length/width)	below middle	at middle	above middle
narrow (high)		 9 linear	
	 2 lanceolate	 8 narrow elliptic	 12 oblanceolate
		 7 medium elliptic	
	 1 triangular	 6 broad elliptic	 11 obovate
medium (medium)		 5 circular	
		 4 narrow oblate	 10 broad obtrullate
broad (low)		 3 medium oblate	

Ad. 8: Only varieties with Leaf: number of divisions: absent or very few: Leaf: shape of apex



1
acute

2
obtuse

3
rounded

4
obcordate

Ad. 9: Only varieties with Leaf: number of divisions: absent or very few: Leaf: longitudinal section

1
concave

3
flat

5
convex

Ad. 10: Only Oakleaf type varieties: Leaf: width of lobes

3
narrow

5
medium

7
broad

Ad. 11: Leaf: anthocyanin coloration
Ad. 12: Leaf: hue of anthocyanin coloration

Anthocyanin coloration (Char. 11)	Hue of anthocyanin coloration (Char. 12)		
	1 reddish	2 purplish	3 brownish
1 absent or very weak	Clarion		
3 weak	Du bon jardinier, Steirer Krauthauptel		Brauner Trotzkopf, Diablo, Maravilla de Verano
5 medium	Lolla rossa		Frisée d'Amérique, Luana, New Red Fire, Salad bowl rossa
7 strong	Jardigon		Duplex, Merveille des quatre saisons
9 very strong	Revolution	Iride	Multired 54

Ad. 13: Leaf: area covered by anthocyanin coloration

Observations should be made on the total area of diffused and/or localised anthocyanin coloration.


3
small


5
medium


7
large


9
very large

Ad. 14: Leaf: colour
Ad. 15: Leaf: intensity of green colour

Only to be observed for green varieties and for two-coloured varieties with 'Leaf: area covered by anthocyanin coloration' less than large (less than note 7 to 9), so the green colour of the leaf can be observed without picking a leaf from the plant.

Intensity of green colour (Char. 15)	Colour (Char. 14)		
	1 green	2 yellowish green	3 greyish green
1 very light			
3 light	Blonde maraîchére, New Red Fire	Lolla Bionda, Steirer Krauthauptel	Celtuce
5 medium	Ballerina	Aquarel, Australische Gele, Dorée de printemps	Clarion, Du bon jardinier, Durango
7 dark	Actarus, Baby Star, Expedition, Verpia		Webbs Wonderful
9 very dark	Pascal, Verdetrix		

Ad. 19: Leaf: size of blisters

Observations should be made on the whole leaf.

3
small

5
medium

7
large

Ad. 20: Leaf: undulation of margin

Observations should be made on undulation of margin of apical part, also apical part in case of divided leaves.

Ad. 21: Leaf: type of incisions of margin

Observations should be made on incisions of the margin at the distal half of the leaf.

1
crenate

2
regularly dentate

3
irregularly dentate

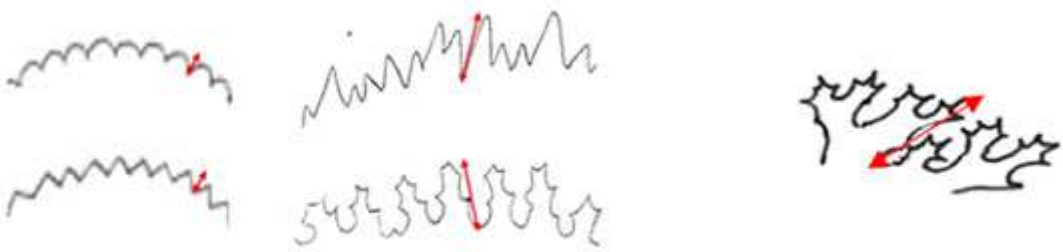
4
bidentate

5
tridentate

Ad. 22: Leaf: depth of incisions of margin

Observations should be made on incisions of the margin at the distal half of the leaf. For varieties with irregularly dentate, bidentate or tridentate incisions describe the deepest incisions and use Char. 23 for the secondary incisions.

The following drawings illustrate how to observe this characteristic for the different types of incisions.



Ad. 23: Only varieties with Leaf: type of incisions of margin: irregularly dentate, bi- or tridentate: Leaf: depth of secondary incisions of margin

Observations should be made on secondary incisions of the margin at the distal half of the leaf. In case of tridentate incisions observations should not be made on tertiary incisions of the margin (the shallowest ones).

Ad. 24: Leaf: density of incisions of margin

Observations should be made on all incisions of the margin at the distal half of the leaf, so in case of irregularly dentate or bidentate both primary and secondary incisions, in case of tridentate also tertiary incisions.

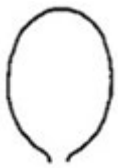
Ad. 25: Leaf: venation

1
not flabellate

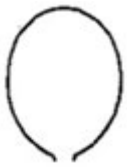
2
semi-flabellate

3
flabellate

Ad. 27: Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Head: shape in longitudinal section

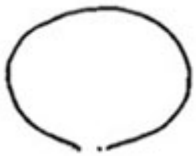


1
narrow elliptic



2
broad elliptic

3
circular



4
narrow oblate

Ad. 29: Only Stem type varieties: Stem: length

length

Ad. 30: Only Stem type varieties: Stem: width

Observations should be made on the broadest part of the stem

width

Ad. 31: Only Stem type varieties: Stem: shape in longitudinal section

1
cylindrical

2
conical

3
fusiform

Ad. 35: Time of beginning of bolting

Observations should be made in a trial with more than 12 hours of day light as lettuce varieties need a long photo period to induce bolting.

Observations should be made when 50% of the plants start to bolt. The top of the bolting stem can be seen or felt at the top of the plant.

Ad. 36: Axillary sprouting

Formation of secondary sprouts beside the main head. Arrow points at one of the secondary sprouts. Observations should be made in overripe stage, just before bolting.

Ad. 37: Bolting stem: fasciation

Observations should be made on the stem of bolted plants after the first flowers are open. For varieties with very late time of beginning of bolting and with strong degree of overlapping of leaves, the cover leaves of the head may be incised just before deterioration in order to be able to observe fasciation.

1
absent or very weak

3
weak

5
medium

7
strong

9
very strong

Ad. 38 to 46: Resistance to *Bremia lactucae* (Bl), several EU isolates

1.	Pathogen	*Bremia lactucae*
2.	Quarantine status	no
3.	Host species	lettuce - *Lactuca sativa* L.
4.	Source of inoculum	GEVES[1] (FR) or Naktuinbouw[2] (NL)
5.	Isolate	Bl: 29EU, Bl: 30EU, Bl: 31EU, Bl: 33EU, Bl: 35EU, Bl: 36EU, Bl: 38EU, Bl: 39EU and Bl: 40EU
6.	Establishment isolate identity	test on differentials (see table below)
7.	Establishment pathogenicity	test on susceptible varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	lettuce plantlets

[1] matref@geves.fr
[2] resistentie@naktuinbouw.nl

8.2	Multiplication variety	susceptible variety, for example Green Towers. For higher isolates, 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	at least 20
9.2	Number of replicates	-
9.3	Control varieties	(informative) differentials (see table below)
9.4	Test design	-
9.5	Test facility	climate room
9.6	Temperature	15°C-18°C
9.7	Light	adequate for good plant growth; seedlings should not etiolate. option: reduced light 24 hours after inoculation
9.8	Season	-
9.9	Special measures	plants may grow on wet blotting paper with or without a nutrient solution, on sand, or on potting soil (see point 13). 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 3.104-1.105
10.3	Plant stage at inoculation	cotyledon stage
10.4	Inoculation method	spraying till run-off option: reduced light 24 hours after inoculation
10.5	First observation	beginning of sporulation on susceptible varieties (around 7 days after inoculation)
10.6	Second observation	3-4 days after first observation (around 10 days after inoculation)
10.7	Final observations	14 days after inoculation two of these three observations may be sufficient; the third notation is optional for observation of evolution of symptoms in case of doubt. 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	resistant 0 no sporulation, no necrosis 1 no sporulation, necrosis present 2 weak sporulation (much less than susceptible control) with necrosis 3 weak sporulation (less than susceptible control and not evolving between second and third observations) with necrosis 4 very sparse sporulation (not evolving between second and third observation) without necrosis susceptible 5 reduced sporulation (compared to susceptible control) without necrosis 6 normal sporulation without necrosis
11.3	Validation of test	on standards. In case of normal sporulation (same level as susceptible control) with necrosis, another test on bigger plants or other substrate must be undertaken.
12.	Interpretation of data in terms of UPOV characteristic states	class 0, 1, 2, 3 and 4: resistant class 5 and 6: susceptible

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. The sowing on soil can be used to see necrosis, but weak sporulation (much less than susceptible control) can appear; when testing on sand, spores can be confused with grains of sand. In case of use of nutritive solution on blotting paper, a fungicide can be added to avoid contamination by saprophytes.

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 https://worldseed.org/our-work/disease-resistance/other-initiatives/ibeb/. The table for isolates mentioned in this protocol and illustrations for the observation scale are given.

	GreenTowers	Dandie	R4T57D	UC Dm14	NunDm15	CGDm16	Colorado	FrRsal-1	Argelès	RYZ 2164	RYZ910457	Bedford	Balesta	Bartoli	Design	Kibrille	Fenston	Bataille	RYZ20007	Set	D sextet code
		Dm3	Dm4	Dm14	Dm15	Dm16	Dm18	Rsal-1	R38	Dm24/38	R52	R53	R54	R55	R56	Dm11,R57	R65	R59	Dm11,R58		
ID	0	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18		
Sextet value		1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16	32		
BI: 7US	+	+	-	+	+	+	+	-	-	-	-		-	-		-	-	-	-	D	61-.-.
BI: 8US	+	+	+	+	+	+	+	-	-	-	-		-	-	+	-	-	+	-	D	63-.-18
BI: 9US	+	+	-	+	+	+	+	+	-	-	+	(-)	-	-	+	-	-	-	-	D	61-09-02
BI: 29EU	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	D	62-07-00
BI: 30EU	+	-	+	+	+	-	+	-	+	+	-	-	-	-	+	-	-	-	-	D	46-06-02
BI: 31EU	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-	-	-	-	D	39-12-02
BI: 33EU	+	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	D	62-07-06
BI: 35EU	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	D	62-15-06
BI: 36EU	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	D	55-15-01
BI: 38EU	+	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	-	-	+	D	46-15-38
BI: 39EU	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-	-	+	D	55-15-33
BI: 40EU	+	-	+	+	+	+	+	+	+	+	+	+	-	+	(-)	-	-	-	-	D	62-31-01

Legend: (+) indicates slightly reduced sporulation, (-) indicates no sporulation with necrosis or very weak sporulation, as defined in the harmonized scale given in Fig. 1.

Ad. 47: Resistance to *Lettuce Mosaic Virus* (LMV) pathotype II
Resistance to pathotype II to be tested in a bio-assay (method i) and/or in a DNA marker test (method ii).

(i) Bio-assay

1.	Pathogen	*Lettuce mosaic virus*
2.	Quarantine status	no
3.	Host species	lettuce - *Lactuca sativa* L.
4.	Source of inoculum	GEVES[3] (FR) or Naktuinbouw[4] (NL)
5.	Isolate	pathotype II (isolates LMV-0 and Ls1 belong to the same pathotype)
6.	Establishment isolate identity	resistant and susceptible controls
7.	Establishment pathogenicity	susceptible control inoculation
8.	Multiplication inoculum	
8.2	Multiplication variety	susceptible control
8.3	Plant stage at inoculation	2-3 leaves
8.4	Inoculation medium	0,05 M PBS, 0,25% (w/v) Na_2SO_3 0,5% $C_5H_{10}NNaS_2.3H_2O$, 4% carborundum and 5% active charcoal
8.5	Inoculation method	rubbing; optionally repeat after 4 d; 1-2 h high humidity after inoculation
8.6	Harvest of inoculum	homogenized fresh leaf in buffer (50% w/v); freeze-dried leaves can be kept less than 1 year in storage, long term storage at -80°C
8.7	Check of harvested inoculum	compare with mock inoculation with LMV buffer + carborundum + charcoal
8.8	Shelf life/viability inoculum	2 h at 4°C or on ice
9.	Format of the test	
9.1	number of plants per genotype	at least 20
9.2	number of replicates	1
9.3	Control varieties	susceptible: Bijou (red), Hilde II (green), Sprinter (green), Sucrine (green) resistant: Capitan (green), Corsica (green), Multired 80 (red)
9.4	Test design	several mock-inoculated plants in the same tray
9.5	Test facility	climate chamber
9.6	Temperature	after inoculation 15-22°C
9.7	Light	12-16 h light ca. 5000 lux
10	Inoculation	
10.1	Preparation inoculum	fresh leaf ground in fresh LMV buffer incl. carborundum and active charcoal
10.3	Plant stage at inoculation	1st leaf well-developed at 1st inoculation, optionally 4 days later 2nd inoculation
10.4	Inoculation method	rubbing, rinse carborundum off
10.7	Final observations	21 days post inoculation
11.	Observations	
11.1	Method	visual estimate of mosaic severity; compare with standards, preferably with standards of same growth type.
11.2	Observation scale	resistant = no symptoms susceptible = growth retardation, young leaves with mosaic, leaf curling
11.3	Validation of test	standards should conform to description
12.	Interpretation of data in terms of UPOV characteristic states	classify resistant or susceptible per plant, see 11.2.
13.	Critical control points	Sprinter is less susceptible than many other susceptible varieties, this variety can be used to detect low inoculation pressure in a specific experiment. anthocyanin coloration in leaves may mask mosaic symptoms and an earlier observation date for green varieties may be possible, depending on the reaction of the standard varieties in the test.

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(ii) DNA marker test

Recessive gene *mo1* (with its alleles *mo1*^{1} or *mo1*^{2}) gives resistance to LMV pathotype II. Resistant alleles mo1^1 and mo1^2 and the presence of the susceptible allele mo1^0 can be detected by the co-dominant marker as described by V. Nicaise *et al* (2003). Specific aspects:

1.	Pathogen	*Lettuce mosaic virus* pathotype II
2.	Functional gene	*mo1* (with two recessive alleles for resistance *mo1*^{1} and *mo1*^{2} and one dominant allele for susceptibility *mo1*^{0})
3.	Probes and primers for Taqman PCR	
3.1.	Assay 1	to distinguish *mo1*^{1} genotypes from *mo1*^{0} and *mo1*^{2} genotypes (6 base deletion at nucleotide position 344-349):

Probe	DNA sequence '5-'3	Fluorophore color (optional)
Pr-del-mo1	GGCTCAAGGAGCTGACTTCTATTG	Texas Red (Susceptible)
Pr-del-mo1^1	GGCTCATGACTTCTATTG	6FAM-MGB (Resistant *mo1*^{1})

Primers	DNA sequence '5-'3
Fw-del-mo1	CAACAACATACATCGACCAA
Rev-del-mo1	CTTCCCACTTAGGCTCGAT

Sequence amplicon: '5-'3
The amplicon sequence of the *mo1*^{0} and *mo1*^{2} allele:
TTACAACAACATACATCGACCAAGCAAGTTGGCTCAAGGAGCTGACTTCTATTGTTTCAAGAATAAAATCGAGCCTAAGTGGGAAGACC
The amplicon sequence of the allele for resistance *mo1*^{1}:
TTACAACAACATACATCGACCAAGCAAGTTGGCTCATGACTTCTATTGTTTCAAGAATAAAATCGAGCCTAAGTGGGAAGACC

3.2.	Assay 2	to distinguish *mo1*^{2} genotypes from *mo1*^{0} and *mo1*^{1} genotypes (SNP at nucleotide position 228):

Probe	DNA sequence '5-'3	Fluorophore color (optional)
Pr-SNP228-*mo1*	CTCCCTCT**G**CTAAGTC	6FAM-MGB (Susceptible)
Pr-SNP228-*mo1*^{2}	ACTCCCTCT**C**CTAAGT	VIC-MGB (Resistant *mo1*^{2})

Primers	DNA sequence '5-'3
Fw-SNP228-*mo1*	GCATCCGCTCGAGCATTC
Rev-SNP228-*mo1*	CTACCCCAAGCGACTTGCTT

Sequence amplicon: '5-'3
The amplicon sequence of the *mo1*^{0} and the *mo1*^{1} allele:
TCAGCATCCGCTCGAGCATTCTTGGACTTTCTGGTTCGATACTCCCTCT**G**CTAAGTCCAAGCAAGTCGCTTGGGGTAGTTCCATGCGCC
The amplicon sequence of the allele for resistance *mo1*^{2}:
TCAGCATCCGCTCGAGCATTCTTGGACTTTCTGGTTCGATACTCCCTCT**C**CTAAGTCCAAGCAAGTCGCTTGGGGTAGTTCCATGCGCC

4.	Format of the test	
4.1	Number of plants per genotype	at least 20 plants
4.2	Control varieties	Homozygous allele for susceptibility *mo1*^{0} present: Sprinter, Sucrine Homozygous allele for resistance *mo1*^{1} present: Capitan, Kanaryole Homozygous allele for resistance *mo1*^{2} present: Corianas Mix DNA to have heterozygous controls
5.	Preparation	
5.1	Preparation DNA	Harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol.
5.2	Preparation PCR	Pipette each DNA sample and a commercial real-time PCR mastermix into individual wells for assay 1 and for assay 2. Analyze the samples in a real-time PCR machine capable of reading the fluorophores of all the probes, with reaction conditions suitable for the mastermix used.

6.	PCR conditions	(detailed test protocol available through Naktuinbouw[5] (NL))

Assay 1:

	Temperature	Time	Ramping speed
Initial activation of enzyme	95°C	2' 00"	
40 cycles	95°C	0' 15"	5˚C/sec
	65°C	0' 48"	5˚C/sec

Assay 2:

	Temperature	Time	Ramping speed
	95°C	2' 00"	
40 cycles	95°C	0' 15"	5˚C/sec
	60°C	0' 48"	5˚C/sec

Analysis at end point RFU.

7.	Observations	
7.1	Observation scale	

Assay 1:

Signal giving Fluorophore		
FAM (*mo1*[1])	Texas Red (*mo1*[0] or *mo1*[2])	
-	x	Homozygous *mo1*[0] or *mo1*[2], or heterozygous *mo1*[0]*mo1*[2]
x	-	Homozygous *mo1*[1]
x	x	Heterozygous *mo1*[0]*mo1*[1] or *mo1*[1]*mo1*[2]
-	-	No result, repeat test

Assay 2:

Signal giving Fluorophore		
FAM (*mo1*[0] or *mo1*[1])	VIC (*mo1*[2])	
(x) (FAM RFU << VIC RFU)	x	Homozygous *mo1*[2]
x	-	Homozygous *mo1*[0] or *mo1*[1], or heterozygous *mo1*[0]*mo1*[1]
x	(x) (FAM RFU >> VIC RFU)	Heterozygous *mo1*[0]*mo1*[2] or *mo1*[1]*mo1*[2]
-	-	No result, repeat test

7.2	Validation of the test	Control varieties should give the expected results.
8.	Interpretation of data in terms of UPOV characteristic states	The combination of the two PCR assays leads to the following predicted result in a bio-assay with LMV pathotype II:

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			Assay 2 (*mo1*^{2})		
			absent	**present homozygous**	**heterozygous**
	Assay 1 (*mo1*^{1})	**absent**	susceptible (*mo1*^{0})	resistant (*mo1*^{2})	susceptible (*mo1*^{0}/*mo1*^{2})
		present homozygous	resistant (*mo1*^{1})	-	-
		heterozygous	susceptible (*mo1*^{0}/ *mo1*^{1})	-	not yet validated
		Heterozygous plants (*mo1*^{0}*mo1*^{1} or *mo1*^{0}*mo1*^{2}) are predicted to be susceptible in the bio-assay, as *mo1*^{1} and *mo1*^{2} are recessive alleles. Heterozygous plants *mo1*^{1}*mo1*^{2} need a conclusion from a bio-assay. Varieties showing a mixture of genotypes (heterozygous plants *mo1*^{0}*mo1*^{1}, *mo1*^{0}*mo1*^{2} or homozygous *mo1*^{0} plants (susceptible predicted phenotype) and homozygous *mo1*^{1} or *mo1*^{2} plants (resistant predicted phenotype)) are predicted to be non-uniform in the bio-assay. In case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the variety is resistant due to on another mechanism.			

Ad. 48: Resistance to *Nasonovia ribisnigri* (Nr) biotype Nr: 0

1.	Pathogen	*Nasonovia ribisnigri*
2.	Quarantine status	no
3.	Host species	lettuce - *Lactuca sativa* L.
4.	Source of inoculum	Naktuinbouw[6] (NL)
5.	Isolate	Nr: 0, preferably red coloured biotype
6.	Establishment isolate identity	the ends of the legs are black, size 1.5-2.5 mm
7.	Establishment pathogenicity	with susceptible control Abel or Green Towers
8.	Multiplication inoculum	
8.2	Multiplication variety	Abel or Green Towers
8.3	Plant stage at inoculation	4 to 6 leaves
8.5	Inoculation method	transfer ~5 aphids per plant
8.6	Harvest of inoculum	transfer to Petri-dish; shake off when aphids are numerous carefully remove aphids using a fine painting brush when only few are available
8.7	Check of harvested inoculum	check the black ends of the aphids legs
8.8	Shelf life/viability inoculum	a few hours in shadow
9.	Format of the test	
9.1	number of plants per genotype	at least 20
9.2	number of replicates	no
9.3	Control varieties	susceptible: Abel, Green Towers, Nadine resistant: Barcelona, Bedford, Dynamite, Silvinas
9.4	Test design	
9.5	Test facility	glasshouse
9.6	Temperature	after inoculation: 20-22°C, keep below 26°C
9.7	Light	daylight
9.9	Special measures	containment of winged aphids needs special attention
10.	Inoculation	
10.1	Preparation inoculum	transfer by shake-off or with brush into Petri-dish
10.3	Plant stage at inoculation	2 to 3 week old seedlings
10.4	Inoculation method	transfer 5 small or medium sized aphids to each plant
10.7	Final observations	15 to 20 days post inoculation

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11.	Observations	
11.1	Method	count red aphids per plant; if many aphids are present, strong growth reduction can be observed; for this observation, a separate aphid free tent is necessary for blanks
11.2	Observation scale	0 no aphids 1 1-5 aphids 2 6-10 aphids 3 >10 aphids
11.3	Validation of test	controls should be >95% ok; if >5% plants are in class 2 or off-type, the experiment should be repeated
12.	Interpretation of data in terms of UPOV characteristic states	0 or 1 resistant 3 susceptible
13.	Critical control points	allow sufficient time for the aphids born after inoculation to mature and turn red; as soon as this is the case, the test must be concluded; this may be before 15 days post inoculation. only adult, red aphids are counted; young aphids are transparent and do not count

Ad. 49: Resistance to *Fusarium oxysporum* f.sp. *lactucae* (Fol) race 1

1.	Pathogen	*Fusarium oxysporum* f.sp*. lactucae*
2.	Quarantine status	EPPO alert list
3.	Host species	lettuce - *Lactuca sativa* L.
4.	Source of inoculum	NIAS Genebank[7] (JP), CREA-SCS[8] (IT), Naktuinbouw[9] (NL), GEVES[10] (FR)
5.	Isolate	Fol: 1
6.	Establishment isolate identity	use microscope and inoculation to lettuce susceptible standard
7.	Establishment pathogenicity	use lettuce susceptible standard
8.	Multiplication inoculum	
8.1	Multiplication medium	inoculation by sowing on contaminated soil: Wheat bran-soil medium inoculation by soaking seedlings: on synthetic liquid medium (e.g. Potatoes Dextrose Broth)
8.6	Harvest of inoculum	inoculation by sowing on contaminated soil: 7-10 day-old culture inoculation by soaking seedlings: 15 days
9.	Format of the test	
9.1	Number of plants per genotype	at least 30, in case of doubt 60
9.2	Number of replicates	at least 2
9.3	Control varieties	susceptible: Cobham Green, Patriot (Cobham Green is slightly less susceptible than Patriot) moderately resistant: Affic, Fuzila, Natexis (Natexis is the lower level of moderate resistance) resistant: Costa Rica No.4, Romasol
9.4	Test design	include control varieties
9.5	Test facility	greenhouse or climate room
9.6	Temperature	25-28°C (day) / 20°C (night)
9.7	Light	under natural day length

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10. Inoculation — two methods can be used for inoculation:

		sowing seeds on contaminated soil	**soaking seedlings**
10.1	Preparation inoculum	wheat bran-soil medium culture mixed with sterilized soil	soaking of roots and of hypocotyl axis for 5 to 15 min in the inoculum suspension
10.2	Quantification inoculum	soil: culture = 20 : 1	spores are harvested and adjusted to 10^6 to 10^7 sp/ml
10.3	Plant stage at inoculation	seeds stimulated to emerge (remark: avoid seeds rotted by factors other than pathogen)	cotyledons to 2 or 3 leaves appearing

10.4 Inoculation method — two methods can be used, as described above

10.5 First observation — 7- 10 days post inoculation

10.6 Second observation — 14 days post inoculation

10.7 Final observations — 20-25 days post inoculation (sowing or soaking). One or two of these 3 observations may be sufficient. The observation for inoculation by soaking is destructive since stems are cut for the observation of vessels.

11. Observations

11.1 Method — visual and/or counting number of plants with symptom; as information calculate a disease index.

11.2 Observation scale

inoculation by sowing seeds on contaminated soil	**inoculation by soaking seedlings**
0: healthy	0: plant without symptoms and healthy vessels 
1: slightly stunting, growing reduction	1: plant with brown vessels only below the cotyledon without yellowing and wilting 
2: severely stunting	2: plant with brown vessels above the cotyledon, without yellowing and wilting 
3: dead plant	3: plant yellowing and wilting, brown vessels 
	4: dead plant 

11.3	Validation of test	results should be compared with results of controls and are depending of the aggressiveness of the test and the distribution of the plants over the categories. a disease index may be helpful (example for the method of inoculation by soaking seedlings: DI= (0A + 1B + 2C + 3D + 4E) / (A + B + C + D + E), where A to E are number of plants in each category).
12.	Interpretation of data in terms of UPOV characteristic states	compare the distribution over the categories with the result of the controls.

Ad. 50: Resistance to *Fusarium oxysporum* f.sp. *lactucae* (Fol) Race 4

1.	Pathogen	*Fusarium oxysporum* f.sp. *lactucae*
2.	Quarantine status	NO : EPPO alert list
3.	Host species	lettuce - *Lactuca sativa* L.
4.	Source of inoculum	e.g. GEVES[11] (FR)
5.	Isolate	Fol: 4, e.g. isolate 04750888 reference strain validated in an interlaboratory test[12] Other validated isolates may be used, as long as producing the same results on the differential set
6.	Establishment isolate identity	The most recent table of differential set is available through ISF at https://worldseed.org/our-work/disease-resistance/differential-hosts/
7.	Establishment pathogenicity	use lettuce susceptible standard
8.	Multiplication inoculum	
8.1	Multiplication medium	on liquid medium (e.g. Potatoes Dextrose Broth)
8.6	Harvest of inoculum	15 days
9.	Format of the test	
9.1	Number of plants per genotype	at least 30 plants, in case of doubt 60 plants
9.2	Number of replicates	at least 2 replicates
9.3	Control varieties	susceptible: Gisela moderately resistant: Ballerina and Patriot (Ballerina is the lowest level of moderate resistance) resistant: Lomeria, Palmos
9.4	Test design	include control varieties
9.5	Test facility	greenhouse or climate room
9.6	Temperature	23-26°C
9.7	Light	under natural day length
10.	Inoculation	
10.1	Preparation inoculum	spores are harvested
10.2	Quantification inoculum	adjust to 10^6 sp/ml
10.3	Plant stage at inoculation	cotyledons to 2 or 3 leaves appearing
10.4	Inoculation method	soaking roots and hypocotyl for 5 to 15 min in the inoculum suspension
10.5	First observation	-
10.6	Second observation	-
10.7	Final observations	20-25 days post inoculation
11.	Observations	
11.1	Method	visual and/or counting number of plants with symptom.
11.2	Observation scale	

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[12] See ISF EG DRT Fol: 4 resistance in lettuce https://worldseed.org/document/isf-final-report-fol-race-4-lettuce/

inoculation by soaking seedlings
0: plant without symptoms and healthy vessels 
1: plant with brown vessels only below the cotyledon without yellowing and wilting 
2: plant with brown vessels above the cotyledon, without yellowing and wilting 
3: plant yellowing and wilting, brown vessels 
4: dead plant 

11.3 Validation of test results should be compared with results of controls and are depending of the aggressiveness of the test and the distribution of the plants over the categories

12 Interpretation of data in terms of UPOV characteristic states compare the distribution over the categories with the result of the controls.

8.3 Lettuce types

See also 5.3 for a table to determine the type using several characteristics.

Butterhead type

Heading; thin to rather thick, tender leaves with a clear midrib; leaf shape circular to transverse broad elliptic; in general no incised margin; head shape ranging from broad elliptic to transvers elliptic

Novita type

Cross between Butterhead and Iceberg type for glasshouse growing. Open heading; leaf structure like Butterhead, incisions of the margin as Iceberg

Iceberg type

Heading with strong or very strong overlapping of upper part of leaves; thick and crispy leaves, predominantly green and greyish green, leaf margin hardly to rather strongly incised, no clear midrib but with flabellate venation

Batavia type

Open to strong heading; generally medium thick, rather strongly blistered leaves, predominately yellowish or medium green; leaf margin with weak to strong undulation

Frisée d'Amérique type

Non-heading, loose, generally quite large plant; thin leaves. Compared to Lollo type in general less undulating margin and showing more leaf blade. Compared to Batavia type, leaves are thinner. Mainly used for baby leaf production

Lollo type

Non-heading; thin leaves with strongly undulated leaf margin. The plant as a whole shows mainly the undulating leaf margins. In general, strongly blistered leaves, blisters are rather small.

Oakleaf type

Thin, divided leaves; divisions have an oakleaf or lobed shape with in general a rounded tip. Radichetta or Catalogna with acute tip of the division. Heart can be loose to dense

Multi-divided type

Non-heading; thin, medium to very strong divided leaves. Tip of divisions can be undulated and incised. Plant may look as a Lollo type, but leaves are always divided

Frillice type

Non-heading; thick, crispy leaves, sometimes weakly divided. Clearly incised leaf margin

Cos type

Elongated and rather tough leaves with a clear midrib, head shape in longitudinal section elliptic, length of head >1.5 x diameter; heading can be very late

Tough 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 type. Suitable for semi-arid conditions

Gem type

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.

Stem type

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/013/6-Rev.4 – *Lactuca sativa* L. – lettuce