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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

Lettuce

UPOV Code: LACTU_SAT

Lactuca sativa L.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by (an) expert(s) from the Netherlands

to be considered by the

*Technical Working Party for Vegetables
 at its forty-ninth session
 to be held in Angers, France
 from 2015-06-15
 to 2015-06-19*

Alternative Names: [*]				
<i>Botanical name</i>	<i>English</i>	<i>French</i>	<i>German</i>	<i>Spanish</i>
Lactuca sativa L.	Lettuce	Laitue	Salat	Lechuga

The purpose of these guidelines (“Test Guidelines”) is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These Test Guidelines should be read in conjunction with the General Introduction and its associated TGP documents.

^{*} These names were correct at the time of the introduction of these Test Guidelines but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Website (www.upov.int), for the latest information.]

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Lactuca sativa* L..

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of seed.

2.3 The minimum quantity of plant material, to be supplied by the applicant, should be:

15,000 seeds

The seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority. In cases where the seed is to be stored, the germination capacity should be as high as possible and should be stated by the applicant.

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 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.

3. Method of Examination

3.1 *Number of Growing Cycles*

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

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".

3.3 *Conditions for Conducting the Examination*

3.3.1 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*

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 *Distinctness*

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

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 Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.1.4 Number of Plants / Parts of Plants to be Examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 20 plants or parts taken from each of 20 plants and any other observations made on all plants in the test, disregarding any off-type plants.

4.1.5 Method of Observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the second 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. color 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 these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

4.2.2 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 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

5. Grouping of Varieties and Organization of the Growing Trial

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

- (a) Seed: color (characteristic 1)
- (b) Leaf: anthocyanin coloration (characteristic 11)
- (c) Time of beginning of bolting under long day conditions (characteristic 32)
- (d) Resistance to downy mildew ("Bremia lactucae") isolate BI: 16 (characteristic 34)
- (e) Resistance to downy mildew ("Bremia lactucae") isolate BI: 29 (characteristic 44)

In the first place, the collection should be divided according to growth types as mentioned in table (a) provided in Chapter 8.1.

In cases of doubt to which growth type a variety belongs to, it should be tested in all relevant growth types.

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction and document TGP/9 "Examining Distinctness".

6. Introduction to the Table of Characteristics

6.1 *Categories of Characteristics*

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

6.2 States of Expression and Corresponding Notes

6.2.1 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.

6.2.2 In the case of qualitative and pseudo-qualitative characteristics (see Chapter 6.3), 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

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

6.3 Types of Expression

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 Example Varieties

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

6.5 *Legend*

- (*) Asterisked characteristic – see Chapter 6.1.2
- QL Qualitative characteristic – see Chapter 6.3
- QN Quantitative characteristic – see Chapter 6.3
- PQ Pseudo-qualitative characteristic – see Chapter 6.3

- MG, MS, VG, VS – see Chapter 4.1.5

(a)-(c) See Explanations on the Table of Characteristics in Chapter 8.

(+) See Explanations on the Table of Characteristics in Chapter 8.

7. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
1. (*) QL VG (a)					
Seed: color					
white				Verpia	1
yellow				Durango	2
black				Kagranner Sommer 2	3
<hr/>					
2. (*) QN MS VG (a) (b)					
Plant: diameter	Plante : diamètre	Pflanze: Durchmesser	Planta: diámetro		
very small	très petit	sehr klein	muy pequeño	Tom Thumb	1
small	petit	klein	pequeño	Gotte à graine blanche	3
medium	moyen	mittel	medio	Clarion, Verpia	5
large	grand	groß	grande	Great Lakes 659	7
very large	très grand	sehr groß	muy grande	El Toro	9
<hr/>					
3. (*) PQ VG (+) (a) (b)					
Plant: head formation					
no head				Blonde à couper améliorée, Lollo rossa, Redair	1
open head				Actarus, Aquarel	2
closed head				Clarion, Roxette	3
<hr/>					
4. QN MG VG (+) (a) (b)					
Only varieties with no head: Plant: number of leaves					
few				Lollo rossa	1
medium				Muraï	3
many				Felucca, Sartre, Xandra	5
<hr/>					

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
5. QN VG (+) (a)					
(c)					
Leaf: attitude	Feuille : port	Blatt: Haltung	Hoja: porte		
erect	dressé	aufrecht	erecto	Feria, Pinokkio	1
semi-erect	demi-dressé	halbaufrecht	semierecto	Faradia, Sartre	3
horizontal	horizontal	waagerecht	horizontal	Divina	5
<hr/>					
6. QN VG (+) (a)					
(c)					
Leaf: number of divisions					
absent or very few				Fiorella, Lollo rossa	1
few				Curletta, Rodagio	3
medium				Ezabel, Jadigon	5
many				Expedition, Multired 54	7
very many				Excite, Ezfrill, Telex	9
<hr/>					
7. QN VG (+) (a)					
(c)					
Only Oakleaf type varieties:					
Leaf: width of divisions					
narrow				Kibrille, Rougini	3
medium				Bandolin, Ribaï	5
broad				Horix, Starix, Vizir	7
<hr/>					
8. PQ VG (+) (a)					
(c)					
Only varieties with divisions absent or very few: Leaf: shape					
narrow elliptic				Verte maraîchère	1
medium elliptic				Xanadu	2
broad elliptic				Amadeus	3
circular				Verpia	4
transverse broad elliptic				Commodore, Fiorella	5
transverse narrow elliptic				Stylist	6
obovate				Raisa	7
broad obtrullate					8
triangular					9

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
9. PQ VG (+) (a) (c) Only varieties with divisions absent or very few: Leaf: shape of apex					
obtuse				Actarus	1
rounded				Blonde maraîchère, Maserati	2
<hr/>					
10. QN VG (+) (a) (c) Only varieties with divisions absent or very few: Leaf: cross section					
concave	concave	konkav	cóncava	Sunstar	1
flat	plate	flach	plana	Clarion, Lollo rossa	3
convex				Tiago	5
<hr/>					
11. (*) QN VG (+) (a) (c) Leaf: anthocyanin coloration					
absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	Clarion	1
weak	faible	gering	débil	Du bon jardinier	3
medium	moyenne	mittel	media	Lollo rossa, Luana	5
strong	forte	stark	fuerte	Merveille des quatre saisons	7
very strong	très forte	sehr stark	muy fuerte	Iride, Revolution	9
<hr/>					
12. (*) PQ VG (+) (a) (c) Leaf: hue of anthocyanin coloration					
reddish				Lollo rossa	1
brownish				Luana, Maravilla de Verano	2
purplish				Faradia, Iride	3

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
13. QN VG (+) (a) (c) Leaf: area covered by anthocyanin coloration					
very small	Feuille : surface couverte par la pigmentation anthocyanique	Blatt: Größe der Anthocyanfärbung	Hoja: área cubierta por la pigmentación antocianica	Steirer Krauthauptel	1
small	petite	klein	pequeña	Diablo	3
medium	moyenne	mittel	media	Luana	5
large	grande	groß	grande	Merveille des quatre saisons	7
very large				Bijou, Revolution	9
<hr/>					
14. (*) PQ VG (+) (a) (c) Leaf: green color					
green				Verpia	1
yellowish green				Dorée de printemps	2
greyish green				Celtuce, Du bon jardinier	3
<hr/>					
15. (*) QN VG (+) (a) (c) Leaf: intensity of green color					
very light	Feuille: intensité de couleur verte	Blatt: Intensität der Grünfärbung	Hoja: intensidad del color verde		1
light	très claire	sehr hell	muy clara	Blonde maraîchère,	3
	claire	hell	clara	Lollo Bionda	
medium	moyenne	mittel	media	Aquarel, Clarion	5
dark	foncée	dunkel	oscura	Expedition, Verpia	7
very dark	très foncée	sehr dunkel	muy oscura	Pascal, Verdetrix	9
<hr/>					
16. QN VG (a) (c) Leaf: glossiness of upper side					
absent or very weak	Feuille: brillance de la face supérieure	Blatt: Glanz der Oberseite	Hoja: brillo de la parte superior		1
weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Divina, Du bon jardinier	
weak	faible	gering	débil	Duplex, Fiorella, Sartre	3
medium	moyenne	mittel	medio	Funnice	5
strong	forte	stark	fuerte	Noisette, Redair	7
very strong	très forte	sehr stark	muy fuerte	Bijou	9
<hr/>					

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
17. QN VG (a) (c)					
Leaf: thickness	Feuille: épaisseur	Blatt: Dicke	Hoja: espesor		
thin	mince	dünn	delgado	Bijou, Lollo rossa, Raisa	3
medium	moyen	mittel	medio	Curtis, Expedition	5
thick	épais	dick	grueso	Frilett, Roxette	7
<hr/>					
18. (*) QN VG (a) (c)					
Leaf: blistering	Feuille : cloûre	Blatt: Blasigkeit	Hoja: abullonado		
absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Duplex, Sartre	1
weak	faible	gering	débil	Fiorella	3
medium	moyenne	mittel	medio	Commodore, Rodagio	5
strong	forte	stark	fuerte	Blonde de Paris, Xanadu	7
very strong	très forte	sehr stark	muy fuerte	Blonde de Doulon, Iride, Karioka	9
<hr/>					
19. QN VG (a) (c)					
Leaf: size of blisters					
small				Dorée de printemps, Faradia, Rodagio	3
medium				Visyon	5
large				Fiorella	7
<hr/>					
20. QN VG (+) (a) (c)					
Leaf: undulation of margin	Feuille : ondulation du bord	Blatt: Randwellung	Hoja: ondulación del borde		
absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	Tiago	1
weak	faible	gering	débil	Commodore	3
medium	moyenne	mittel	media	Noisette, Pentared	5
strong	forte	stark	fuerte	Calmar, Invicta	7
very strong	très forte	sehr stark	muy fuerte	Lollo rossa	9

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
21. QN VG (+) (a)					
(c)					
Leaf: depth of incisions of margin					
absent or very shallow				Actarus, Clarion, Tiago	1
shallow				Pentared, Unicum	3
medium				Santarinas	5
deep				Expedition	7
very deep					9
<hr/>					
22. PQ VG (+) (a)					
(c)					
Leaf: type of incisions of margin					
crenate				Gloire du Dauphiné	1
dentate				Lollo rossa	2
bidentate				Expedition	3
<hr/>					
23. QN VG (+) (a)					
(c)					
Only varieties with type of incisions bidentate: Leaf: depth of secondary incisions of margin					
very shallow					1
shallow				Great Lakes 659	3
medium				Expedition	5
deep					7
<hr/>					
24. QN VG (+) (a)					
(c)					
Leaf: density of incisions of margin					
very sparse					1
sparse				Maravilla de Verano	3
medium				Calmar	5
dense				Grand Rapids	7
very dense				Locarno	9
<hr/>					

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
25. QN VG (+) (a) (c) Leaf: venation					
not flabellate				Verpia, Xanadu	1
semi flabellate				Kibrille, Muraï	2
flabellate				Locarno, Roxette	3
<hr/>					
26. QN MS VG (a) (b) Only varieties with closed head: Head: size					
very small				Tom Thumb	1
small				Xanadu	3
medium				Fiorella, Soraya	5
large				Great Lakes 659	7
very large				Blonde maraîchère, El Toro	9
<hr/>					
27. (*) PQ VG (+) (a) (b) Only varieties with closed head: Head: shape in longitudinal section					
narrow elliptic				Actarus, Verte maraîchère	1
broad elliptic				Amadeus, Sucrine	2
circular				Verpia	3
transverse broad elliptic				Ametist	4
<hr/>					
28. QN VG (+) (a) (b) Only varieties with closed head: Head: degree of overlapping of upper part of leaves					
weak				Auvona, Curtis	3
medium				Augusta, Fiorella	5
strong				Kanaria	7
very strong				Roxette, Vanguard 75	9
<hr/>					

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
29. QN VG (a) (b)					
Head: density					
loose				Nanda	3
medium				Daguan, Delice	5
dense				Atella, Islandia	7
very dense				Rubette	9
<hr/>					
30. QN VG (+)(a)					
Axillary sprouting					
absent or weak				Claridia, Shotter, Valmaine, Xanadu	1
medium				Actarus	2
strong				Amible, Bassoon	3
<hr/>					
31. QN MG VG (a)					
Only varieties with closed head: Time of harvest maturity					
very early				Gotte jaune d'or	1
early				Pantlika, Sucrine	3
medium				Clarion	5
late				Blonde maraîchère, Calmar	7
very late				El Toro, Pinokkio	9
<hr/>					
32. (*) QN MG VG (+)(a)					
Time of beginning of bolting under long day conditions					
very early				Blonde à couper améliorée	1
early				Gotte à graine blanche	3
medium				Pantlika	5
late				Hilde II	7
very late				Erika, Roxette	9
<hr/>					

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
33. QN VG (+)(a)					
Stem: fasciation					
absent or very weak				Aquarel, Gotte à graine blanche	1
weak				Verte maraîchère	3
medium				Amadeus	5
strong				Rougini	7
very strong				Sartre, Verdatrix	9
<hr/>					
34. (*) QL VG (+)					
(a)					
Resistance to downy mildew ("Bremia lactucae") isolate BI: 16					
absent				Green Towers	1
present				Argelès, Ninja	9
<hr/>					
35. QL VG (a)					
Resistance to downy mildew ("Bremia lactucae") isolate BI: 17					
absent				Green Towers	1
present				Argelès, Ninja	9
<hr/>					
36. QL VG (a)					
Resistance to downy mildew ("Bremia lactucae") isolate BI: 20					
absent				Green Towers	1
present				Argelès, Ninja	9
<hr/>					
37. QL VG (a)					
Resistance to downy mildew ("Bremia lactucae") isolate BI: 21					
absent				Green Towers	1
present				Argelès, Colorado	9
<hr/>					

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
38. QL VG (a) Resistance to downy mildew ("Bremia lactucae") isolate BI: 22					
absent				Green Towers	1
present				Discovery, Ninja	9
<hr/>					
39. QL VG (a) Resistance to downy mildew ("Bremia lactucae") isolate BI: 23					
absent				Green Towers	1
present				Colorado, Discovery, Ninja	9
<hr/>					
40. QL VG (a) Resistance to downy mildew ("Bremia lactucae") isolate BI: 24					
absent				Argelès, Colorado	1
present				Dandie, NunDm15, UCdM14	9
<hr/>					
41. QL VG (a) Resistance to downy mildew ("Bremia lactucae") isolate BI: 25					
absent				Colorado, Discovery	1
present				Argelès, Ninja	9
<hr/>					
42. QL VG (a) Resistance to downy mildew ("Bremia lactucae") isolate BI: 26					
absent				Colorado, Discovery	1
present				Balesta, Bedford	9
<hr/>					
43. QL VG (a) Resistance to downy mildew ("Bremia lactucae") isolate BI: 27					
absent				Balesta, Colorado	1
present				Discovery, Ninja	9
<hr/>					

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<hr/>					
44. (*) QL VG (+)(a) Resistance to downy mildew ("Bremia lactucae") isolate BI: 29					
absent				Argelès, Discovery	1
present				Balesta, Ninja	9
<hr/>					
45. QL VG (a) Resistance to downy mildew ("Bremia lactucae") isolate BI: 30					
absent				Argelès, Colorado	1
present				Balesta, Ninja	9
<hr/>					
46. QL VG (a) Resistance to downy mildew ("Bremia lactucae") isolate BI: 31					
absent				Colorado, RYZ910457	1
present				Argelès, Balesta	9
<hr/>					
47. QL VG (+) (a) Resistance to "lettuce mosaic virus" (LMV) pathotype LMV: 0					
absent				Bijou, Hilde II, Sprinter	1
present				Corsica, Diveria	9
<hr/>					
48. QL VG (+) (a) Resistance to "Nasonovia ribisnigri" biotype Nr: 0					
absent				Abel, Green Towers, Nadine	1
present				Barcelona, Dynamite, Silvinas	9
<hr/>					
49. QN VG (+) (a) Resistance to "Fusarium oxysporum f.sp. lactucae" race 1 susceptible					
moderately resistant				Cobham Green, Patriot	1
highly resistant				Affic, Fuzila, Natexis	2
				Costa Rica No. 4, Romasol	3

8. Explanations on the Table of Characteristics

8.1 *Explanations covering several characteristics*

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

(a)

Plant: growth type	Example varieties	Plant: head formation	Leaf: number of divisions	Leaf: thickness	Leaf: undulation of margin	Leaf: venation	<u>Only varieties with closed head:</u> Head: shape in longitudinal section
Butterhead type	Clarion, Maikönig, Sartre	closed head	absent or very few	thin to thick	absent to weak	not flabellate	circular or transverse broad elliptic
Novita type	Norvick	open head	absent or very few	thin to medium	very weak to medium	flabellate	-
Iceberg type	Great Lakes 659, Roxette, Saladin, Vanguard 75	closed head	absent or very few	thick	absent to medium	flabellate	circular or transverse broad elliptic
Batavia type	Aquarel, Curtis, Funnice, Felucca, Grand Rapids, Masaïda, Visyon	open head or closed head	absent or very few	medium to thick	weak to very strong	flabellate	broad elliptic, circular or transverse broad elliptic
Frisée d'Amérique type	Bijou, Blonde à couper améliorée, Faradia	no head	absent or very few	thin	absent to strong	flabellate or not flabellate or semi	-
Lollo type	Lollo rossa, Revolution	no head	absent or very few	thin	strong to very strong	flabellate	-
Oakleaf type	Catalogna, Kipling, Muraï, Salad Bowl	no head	few to many	thin	absent to weak	flabellate or not flabellate or semi	-
Multi-divided type	Curletta, Duplex, Jadigon, Rodagio	no head	medium to very many	thin	weak to very strong	flabellate	-
Frillice type	Frilett	no head	absent or very few	thick	weak to strong	flabellate	-
Cos type	Actarus, Blonde maraîchère, Pinokkio	closed head	absent or very few	medium to thick	absent to weak	not flabellate	narrow elliptic
Gem type	Craquerelle du Midi, Sucrine, Xanadu	closed head	absent or very few	medium to thick	absent to weak	not flabellate	broad elliptic, circular or transverse broad elliptic
Stem type	Celtuçe	no head	absent or very few	thin to medium	absent to weak	not flabellate	-



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.

Butterhead type



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

Novita 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.

Iceberg type



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

Batavia type



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 babyleaf 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.



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.

Cos type



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

(b) Plant and head: Observations on the plant and head should be made at harvest maturity. For non-heading varieties observations should be made just before deterioration and before bolting.

(c) Leaf: Observations on the leaf should be made at harvest maturity. For varieties with a closed head the largest outer leaves should be observed. For non-heading varieties the largest leaves should be observed, just before deterioration and before bolting.

8.2 Explanations for individual characteristics

Ad. 3: Plant: head formation

- (1) No head: plant with a loose structure of the heart. By cutting the stem out of the harvested plant, the plant will fall apart into loose leaves.
- (2) Open head: plant with a dense structure of the heart. By cutting the stem out of the harvested plant, an open head will remain of which the upper part of leaves are not overlapping.
- (3) Closed head: plant with a dense structure of the heart. By cutting the stem out of the harvested plant, the outer leaves will fall off, but a closed head will remain of which the upper part of leaves are overlapping.



1 - no head



2 - open head



3 - closed head

Ad. 4: Only varieties with no head: Plant: number of leaves

Observations should be made on the whole plant.



1 - few

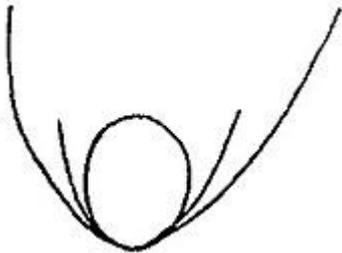


3 - medium

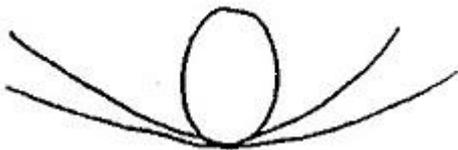


5 - many

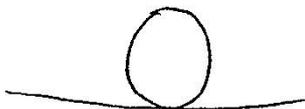
Ad. 5: Leaf: attitude



1 - erect



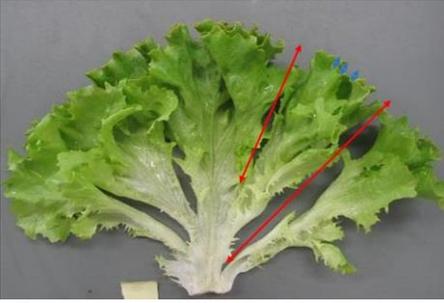
3 - semi-erect



5 - horizontal

Ad. 6: Leaf: number of divisions

To observe only the incisions more than halfway to the midrib at the whole leaf. Incisions less than halfway to the midrib are to be described as incisions of the margin (Char. 21 to 24).



Red arrows: divisions, blue arrows:
incisions (examples)



1 - absent or very few



5 - medium



7 - many



9 - very many

Ad. 7: Only Oakleaf type varieties: Leaf: width of divisions



3 - narrow



5 - medium

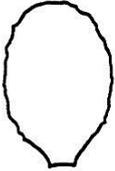


7 - broad

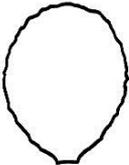
Ad. 8: Only varieties with divisions absent or very few: Leaf: shape



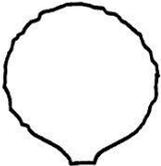
1 - narrow elliptic



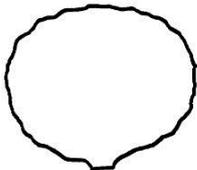
2 - medium elliptic



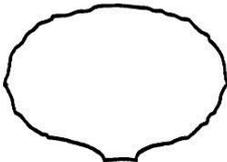
3 - broad elliptic



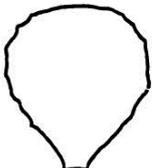
4 - circular



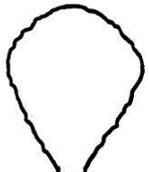
5 - transverse broad elliptic



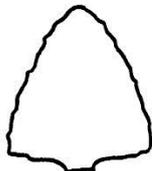
6 - transverse narrow elliptic



7 - obovate



8 - broad obtrullate



9 - triangular

Ad. 9: Only varieties with divisions absent or very few: Leaf: shape of apex

For most lettuce varieties the apex is rounded, curved like the outline of a circle. For some varieties (especially in Cos type) the apex is more angular, to be described as obtuse.

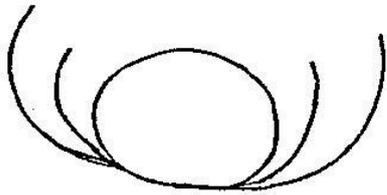


1 - obtuse



2 - rounded

Ad. 10: Only varieties with divisions absent or very few: Leaf: cross section



1 - concave



3 - flat



5 - convex

Ad. 11: Leaf: anthocyanin coloration

Ad. 12: Leaf: hue of anthocyanin coloration

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

Ad. 13: Leaf: area covered by anthocyanin coloration

To observe the total area of diffused and/or localised anthocyanin coloration.

Ad. 14: Leaf: green color

Ad. 15: Leaf: intensity of green color

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

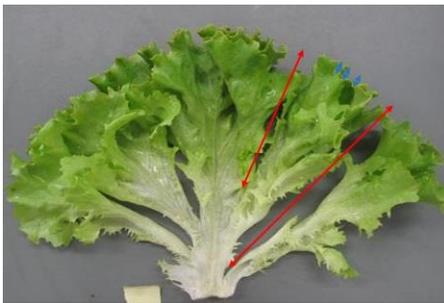
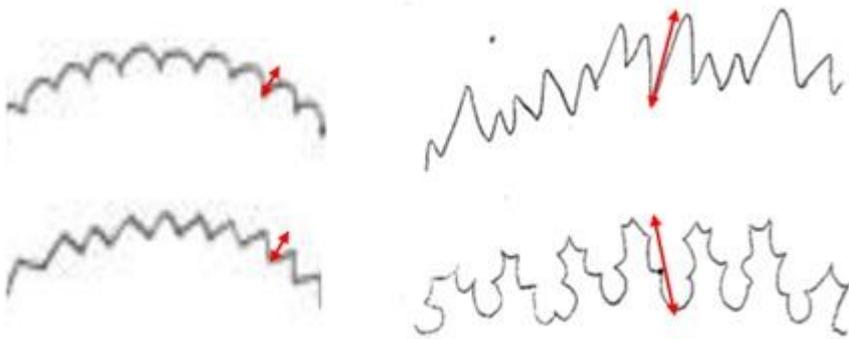
Intensity of green color (Ch. 15)	Green color (Ch. 14)		
	1 absent	2 yellowish	3 greyish
1 very light			
3 light	Blonde maraîchère, New Red Fire	Lollo, Steirer Krauthauptel	Celtuce
5 medium	Ballerina	Aquarel, Australische Gele, Dorée de printemps	Clarion, Du bon jardinier, Durango
7 dark	Actarus, Baby Star, Expedition, Verpia		Webbs Wonderful
9 very dark	Pascal, Verdatrix		

Ad. 20: Leaf: undulation of margin

Observe undulation of margin of apical part; also apical part of divisions in case of divided leaves.

Ad. 21: Leaf: depth of incisions of margin

To observe incisions of the margin at distal half, less than halfway to the midrib. Incisions more than halfway to the midrib are to be described as the number of divisions (Char. 6).
For varieties with bidentate incisions describe the deepest incisions and use Char. 23 for the secondary incisions.



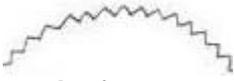
Red arrows: divisions, blue arrows: incisions (examples)

Ad. 22: Leaf: type of incisions of margin

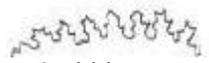
To observe incisions of the margin at distal half, less than halfway to the midrib. Incisions more than halfway to the midrib are to be described as the number of divisions (Char. 6).



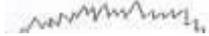
1 - crenate



2 - dentate



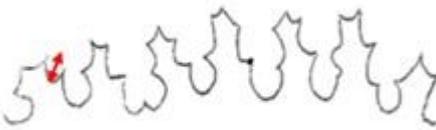
3 - bidentate



3 - bidentate

Ad. 23: Only varieties with type of incisions bidentate: Leaf: depth of secondary incisions of margin

To observe secondary incisions of the margin at distal half.



Ad. 24: Leaf: density of incisions of margin

To observe all incisions of the margin at distal half, less than halfway to the midrib, so in case of bidentate both primary and secondary incisions. Incisions more than halfway to the midrib are to be described as the number of divisions (Char. 6).

Ad. 25: Leaf: venation



1 - not flabellate

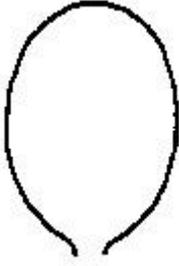


2 - semi flabellate

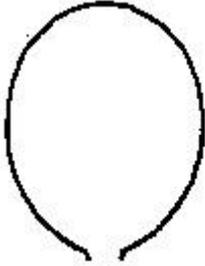


3 - flabellate

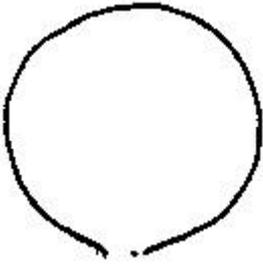
Ad. 27: Only varieties with closed head: Head: shape in longitudinal section



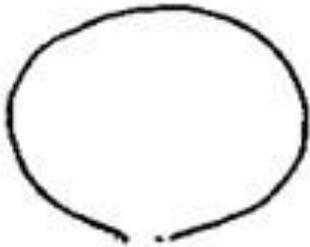
1 - narrow elliptic



2 - broad elliptic



3 - circular



4 - transverse broad elliptic

Ad. 28: Only varieties with closed head: Head: degree of overlapping of upper part of leaves



3 - weak



5 - medium



7 - strong



9 - very strong

Ad. 30: Axillary sprouting

To observe at the start of bolting.

Ad. 32: Time of beginning of bolting under long day conditions

To observe 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. 33: Stem: fasciation

Observations should be made on the stem of bolted plants, not earlier than when the first flowers are open. Varieties with very late time of beginning of bolting and closed head: the cover leaves of the head should be incised just before deterioration in order to be able to observe fasciation.

Ad. 34: Resistance to downy mildew ("Bremia lactucae") isolate BI: 16

Regarding the asterisked characteristics 34 and 44 applies phasing in/phasing out. A period of 5 years after publication of this guideline may be used by members of the Union to develop experience with characteristic 44 Resistance to downy mildew isolate BI: 29.

After this period of 5 years characteristic 44 should always be examined.

After this period of 5 years members of the Union are no longer obliged to examine characteristic 34 (Resistance to downy mildew isolate BI: 16).

1. Pathogen	<i>Bremia lactucae</i>
2. Quarantine status	no
3. Host species	Lettuce - <i>Lactuca sativa</i> L.
4. Source of inoculum	GEVES ¹ (FR) or Naktuinbouw ² (NL)
5. Isolate	BI: 2,5,7,12,14,15,16,17,18,20-27,29-31
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 leaf
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	Normally 60, minimum 20
9.2 Number of replicates	-
9.3 Control varieties	(Informative) differentials (see table below)
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
9.8 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.

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² resistentie@naktuinbouw.nl

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 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

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 isolates mentioned in this guideline is given. Seed of differential varieties can be obtained at GEVES³ (FR) and at Naktuinbouw⁴ (NL).

	GreenTowers	Dandie	R4T57D	UC Dm14	NunDm15	CGDm16	Colorado	FrRsah1	Argelés	RYZ 2164	RYZ910457	Bedford	Bales ta	Bartoli	Design	Kibrille	C sextet code
	Dm3	Dm4	Dm14	Dm15	Dm16	Dm18	Rsa1-1	R38									
Set position	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15		
Sextet value	1	2	4	8	16	32	1	2	4	8	16	32	1	2	4		
BI: 16	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	19-00-00
BI: 17	+	+	-	+	+	-	+	+	-	-	-	(+)	-	-	-	-	45-17-00
BI: 20	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	51-00-00
BI: 21	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	27-01-00
BI: 22	+	-	+	+	+	-	+	-	-	-	-	-	+	-	-	-	46-32-00
BI: 23	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	19-02-00
BI: 24	+	-	+	-	-	+	+	-	+	-	-	-	-	-	(-)	-	50-02-00
BI: 25	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	50-01-00
BI: 26	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	51-03-00
BI: 27	+	+	+	+	+	-	+	-	+	+	-	-	+	-	-	-	47-38-00
BI: 29	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	62-07-00
BI: 30	+	-	+	+	+	-	+	-	+	+	-	-	-	-	+	-	46-06-02
BI: 31	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-	39-12-02

³ matref@geves.fr

⁴ resistentie@naktuinbouw.nl

Ad. 44: Resistance to downy mildew ("Bremia lactucae") isolate BI: 29

Regarding the asterisked characteristics 34 and 44 applies phasing in/phasing out. A period of 5 years after publication of this guideline may be used by members of the Union to develop experience with characteristic 44 Resistance to downy mildew isolate BI: 29.

After this period of 5 years characteristic 44 should always be examined.

After this period of 5 years members of the Union are no longer obliged to examine characteristic 34 (Resistance to downy mildew isolate BI: 16).

Ad. 47: Resistance to "lettuce mosaic virus" (LMV) pathotype LMV: 0

1. Pathogen	Lettuce mosaic virus
2. Quarantine status	No
3. Host species	Lettuce - <i>Lactuca sativa</i> L.
4. Source of inoculum	GEVES ⁵ (FR) or Naktuinbouw ⁶ (NL)
5. Isolate	pathotype LMV: 0 (isolates LMV: 0 and Ls-1 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 ₂ SO ₃ 0,5% C ₅ H ₁₀ NNaS ₂ .3H ₂ O, 4% carborundum and 5% active charcoal
8.5 Inoculation method	rubbing; 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) Resistant: Corsica (green), Diveria (red)
9.4 Test design	8 mock-inoculated plants in the same tray
9.5 Test facility	climate chamber
9.6 Temperature	2 days after sowing 15°C, after 1 st inoculation 22/18°C d/n, after 2nd inoculation again 15°C
9.7 Light	16/8 h d/n; 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 1 st inoculation, 4 days later 2nd inoculation
10.4 Inoculation method	rubbing, rinse carborundum off
10.7 Final observations	21 days post inoculation for red lettuce; 14 days post inoculation for green lettuce
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 R or S per plant, see 11.2.

⁵ service.clients@geves.fr

⁶ resistentie@naktuinbouw.nl

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.

Ad. 48: Resistance to "Nasonovia ribisnigri" biotype Nr: 0

- | | |
|---|--|
| 1. Pathogen | <i>Nasonovia ribisnigri</i> |
| 2. Quarantine status | no |
| 3. Host species | Lettuce - <i>Lactuca sativa</i> L. |
| 4. Source of inoculum | Naktuinbouw ⁷ (NL) |
| 5. Isolate | Nr: 0, preferably red colored 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 |
| 8. Multiplication inoculum | |
| 8.2 Multiplication variety | Abel |
| 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 | minimum 20 |
| 9.2 number of replicates | no |
| 9.3 Control varieties | Susceptible: Abel, Green Towers, Nadine
Resistant: Barcelona, Dynamite, Silvinas |
| 9.4 Test design | |
| 9.5 Test facility | glasshouse |
| 9.6 Temperature | Aater 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 |
| 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 |

⁷ resistantie@naktuinbouw.nl

Ad. 49: Resistance to "Fusarium oxysporum f.sp. lactucae" race 1

1. Pathogen	<i>Fusarium oxysporum</i> f.sp. <i>lactucae</i>
2. Quarantine status	EPPO alert list
3. Host species	<i>Lactuca sativa</i> L.
4. Source of inoculum	NIAS Genebank ⁸ (JP), CRA-SCS ⁹ (IT), Naktuinbouw ¹⁰ (NL), GEVES ¹¹ (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	20 °C (day) / 28 °C (night)
9.7 Light	under natural day length
10. Inoculation	
10.1 Preparation inoculum	<ul style="list-style-type: none">• Inoculation by sowing on contaminated soil: wheat bran-soil medium culture mixed with sterilized soil• Inoculation by soaking seedlings: soaking of roots and of hypocotyl axis for 5 to 15 min in the inoculum suspension and transplantation of inoculated plantlets in soil
10.2 Quantification inoculum	<ul style="list-style-type: none">• Inoculation by sowing on contaminated soil: soil : culture = 20 : 1• Inoculation by soaking seedlings: spores are harvested and adjusted to 10⁶ to 10⁷ sp/ml
10.3 Plant stage at inoculation	<ul style="list-style-type: none">• inoculation by sowing on contaminated soil: seeds stimulated to emerge (remark: avoid seeds rotted by factors other than pathogen)• inoculation by soaking seedlings: cotyledons to 2 or 3 leaves appearing
10.4 Inoculation method	two methods can be used for inoculation: by sowing seeds on contaminated soil or by soaking seedlings
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	<ul style="list-style-type: none">• inoculation by sowing on contaminated soil:

⁸ genebank@nias.affrc.go.jp

⁹ romana.bravi@entecra.it

¹⁰ resistentie@naktuinbouw.nl

¹¹ service.clients@geves.fr

symptoms: stunting, wilting, dead plant

0: healthy

1: slightly stunting, growing reduction

2: severely stunting

3: die

- 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. A disease index may be helpful ($DI = (0A + 1B + 2C + 3D + 4E) / (A + B + C + D + E)$, where A to E are number of plants in each category).

12. Interpretation of data in terms of UPOV characteristic states of Compare the distribution over the categories with the result of the controls. For information a disease index can be used.

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10. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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	Application date: (not to be filled in by the applicant)
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TECHNICAL QUESTIONNAIRE
 to be completed in connection with an application for plant breeders' rights

1. Subject of the Technical Questionnaire			
1.1.1	Botanical Name	Lactuca sativa L.	
1.1.2	Common Name	Lettuce	
1.1.3			

2. Applicant	
Name	<input type="text"/>
Address	<input type="text"/>
Telephone No.	<input type="text"/>
Fax No.	<input type="text"/>
E-mail address	<input type="text"/>
Breeder (if different from applicant)	<input type="text"/>

3. Proposed denomination and breeder's reference	
Proposed denomination (if available)	<input type="text"/>
Breeder's reference	<input type="text"/>

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

- 4.1.1 Crossing [...]
- 4.1.2 Mutation (please state parent variety) [...]
- 4.1.3 Discovery and development
(please state where and when discovered and how developed) [...]
- 4.1.4 Other
(please provide details) [...]

4.2 Method of propagating the variety

4.2.1 Seed-propagated varieties

- (a) Self-pollination
- (b) Other
(please provide details)

.....
:
:
:
.....

4.2.2 Other

(please provide details)

.....
:
:
:
.....

5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).

Characteristics	Example Varieties	Note
5.1 (1) Seed: color		
white	Verpia	1[]
yellow	Durango	2[]
black	Kagraner Sommer 2	3[]
5.2 (11) Leaf: anthocyanin coloration		
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[]
very strong	Iride, Revolution	9[]
5.3 (15) Leaf: intensity of green color		
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, Verdatrix	9[]
5.4 (32) Time of beginning of bolting under long day conditions		
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[]
very late	Erika, Roxette	9[]
5.5 (34) Resistance to downy mildew ("Bremia lactucae") isolate BI: 16		
absent	Green Towers	1[]
present	Argelès, Ninja	9[]
5.6 (44) Resistance to downy mildew ("Bremia lactucae") isolate BI: 29		
absent	Argelès, Discovery	1[]
present	Balesta, Ninja	9[]

6. Similar varieties and differences from these varieties

Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the similar variety(ies)	Describe the expression of the characteristic(s) for your candidate variety
---	---	--	--

Example

Comments:

--

7. Additional information which may help in the examination of the variety

7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

Yes [] No []

(If yes, please provide details)

7.2 Are there any special conditions for growing the variety or conducting the examination?

Yes [] No []

(If yes, please provide details)

7.3 Other information

Growth type (see chapter 8.1 for explanation)

Butterhead type	[]
Novita type	[]
Iceberg type	[]
Batavia type	[]
Frisée d'Amérique type	[]
Lollo type	[]
Oakleaf type	[]
Multi-divided type	[]
Frillice type	[]
Cos type	[]
Gem type	[]
Stem type	[]

8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [] No []

(b) Has such authorization been obtained?

Yes [] No []

If the answer to (b) is yes, please attach a copy of the authorization.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:												
<p>9. Information on plant material to be examined or submitted for examination</p> <p>9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.</p> <p>9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:</p> <table data-bbox="220 562 1326 763"><tr><td>(a) Microorganisms (e.g. virus, bacteria, phytoplasma)</td><td>Yes []</td><td>No []</td></tr><tr><td>(b) Chemical treatment (e.g. growth retardant, pesticide)</td><td>Yes []</td><td>No []</td></tr><tr><td>(c) Tissue culture</td><td>Yes []</td><td>No []</td></tr><tr><td>(d) Other factors</td><td>Yes []</td><td>No []</td></tr></table> <p>Please provide details for where you have indicated "yes".</p> <p>.....</p>			(a) Microorganisms (e.g. virus, bacteria, phytoplasma)	Yes []	No []	(b) Chemical treatment (e.g. growth retardant, pesticide)	Yes []	No []	(c) Tissue culture	Yes []	No []	(d) Other factors	Yes []	No []
(a) Microorganisms (e.g. virus, bacteria, phytoplasma)	Yes []	No []												
(b) Chemical treatment (e.g. growth retardant, pesticide)	Yes []	No []												
(c) Tissue culture	Yes []	No []												
(d) Other factors	Yes []	No []												
<p>10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:</p> <table data-bbox="220 1070 1390 1249"><tr><td data-bbox="220 1070 475 1137">Applicant's name</td><td colspan="2" data-bbox="480 1070 1385 1137"></td></tr><tr><td data-bbox="220 1144 475 1249">Signature</td><td data-bbox="480 1144 962 1249"></td><td data-bbox="975 1144 1390 1249">Date</td></tr></table>			Applicant's name			Signature		Date						
Applicant's name														
Signature		Date												

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