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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

LETTUCE

UPOV Code(s): LACTU_SAT

Lactuca sativa L.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by experts from the Netherlands

to be considered by the

Technical Working Party for Vegetables
at its fiftieth session, to be held in Brno, Czech Republic,
from 2016-06-27 to 2016-07-01

Disclaimer: this document does not represent UPOV policies or guidance

Alternative names:*

Botanical name	*English*	*French*	*German*	*Spanish*
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

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

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 or 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 of plants 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 of seed-propagated varieties, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 60 plants, 2 off-types are allowed.

4.3 Stability

4.3.1 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 (characteristic 35)
(d) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 16 (characteristic 38)

In the first place, the collection should be divided according to types as mentioned in the table below. In cases of doubt to which type a variety belongs to, it should be tested in all relevant types.

Type	Example varieties	Plant: degree of overlapping of upper part of leaves	Leaf: number of divisions	Leaf: thickness	Leaf: undulation of margin	Leaf: venation	Only varieties with degree of overlapping of upper part of leaves medium or strong: Head: shape in longitudinal section
Butterhead type	Clarion, Maikönig, Sartre	medium to strong	absent or very few	thin to thick	absent to weak	not flabellate	circular or transverse broad elliptic
Novita type	Novick	absent or weak	absent or very few	thin to medium	very weak to medium	flabellate	-
Iceberg type	Great Lakes 659, Roxette, Saladin, Vanguard 75	strong	absent or very few	thick	absent to medium	flabellate	circular or transverse broad elliptic
Batavia type	Aquarel, Curtis, Fumnice, Feluca, Grand Rapids, Masaida, Visyon	absent or weak to strong	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	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, Murai, Salad Bowl	absent or weak	few to many	thin	absent to weak	flabellate or not flabellate or semi	-
Multi-divided type	Cutletta, 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 transverse broad elliptic
Stem type	Celtuce, Guasihong	absent or weak	absent or very few	thin to medium	absent to weak	not flabellate	-

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

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1	**2**	**3**	**4**	**5**	**6**	**7**			
		Name of characteristics in English		**Nom du caractère en français**		**Name des Merkmals auf Deutsch**	**Nombre del carácter en español**		
		states of expression		types d'expression		Ausprägungsstufen	tipos de expresión		

1 Characteristic number

2 (*) Asterisked characteristic – see Chapter 6.1.2

3 Type of expression
QL Qualitative characteristic – see Chapter 6.3
QN Quantitative characteristic – see Chapter 6.3
PQ Pseudo-qualitative characteristic – see Chapter 6.3

4 Method of observation (and type of plot, if applicable)
MG, MS, VG, VS – see Chapter 4.1.5

5 (+) See Explanations on the Table of Characteristics in Chapter 8.2

6 (a)-(c) See Explanations on the Table of Characteristics in Chapter 8.1

7 Not applicable

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

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1.	**(*)**	**QL VG**					
		Seed: color					
		white				Verpia	1
		yellow				Durango	2
		black				Kagraner Sommer 2	3
2.	**(*)**	**QN MS/VG**	**(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
3.	**(*)**	**QN VG**	**(+)**	**(a), (b)**			
		Plant: degree of overlapping of upper part of leaves					
		absent or weak				Actarus, Aquarel, Blonde à couper améliorée, Curtis, Lollo rossa	1
		medium				Augusta, Clarion, Fiorella	2
		strong				Roxette, Vanguard 75	3
4.		**QN MS/VG**	**(+)**	**(b), (b)**			
		Only varieties with degree of overlapping of upper part of leaves absent or weak: Plant: number of leaves					
		few				Lollo rossa	3
		medium				Muraï	5
		many				Felucca, Sartre, Xandra	7
5.		**QN VG**	**(+)**	**(c)**			
		Leaf: attitude					
		erect	dressé	aufrecht	erecto	Feria, Pinokkio	1
		semi-erect	demi-dressé	halbaufrecht	semierecto	Expedition, Sartre	3
		horizontal	horizontal	waagerecht	horizontal	Divina	5

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
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
7.		**QN VG**	**(+)**	**(c)**			
		Only Oakleaf type varieties: Leaf: width of lobes					
		narrow				Kibrille, Rougini	3
		medium				Bandolin, Ribaï	5
		broad				Horix, Starix, Vizir	7
8.		**PQ VG**	**(+)**	**(c)**			
		Only varieties with divisions absent or very few: Leaf: shape					
		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
		obovate				Raisa	10
		oblanceolate				Xiangshengcai	11
		broad obtrullate					12

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
9.		**PQ VG**	**(+) (c)**				
		Only varieties with divisions absent or very few: Leaf: shape of apex					
		obtuse				Actarus	1
		rounded				Blonde maraîchère, Maserati	2
		obcordate				PS 6545691	3
10.		**QN VG**	**(+) (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
11.	**(*)**	**QN VG**	**(+) (c)**				
		Leaf: anthocyanin coloration	**Feuille : pigmentation anthocyanique**	**Blatt: Anthocyanfärbung**	**Hoja: pigmentación antociánica**		
		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
12.	**(*)**	**PQ VG**	**(+) (c)**				
		Leaf: hue of anthocyanin coloration					
		reddish				Lollo rossa	1
		brownish				Luana, Maravilla de Verano	2
		purplish				Iride	3

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
13.		**QN VG**	**(+) (c)**				
		Leaf: area covered by anthocyanin coloration	**Feuille : surface couverte par la pigmentation anthocyanique**	**Blatt: Größe der Anthocyanfärbung**	**Hoja: área cubierta por la pigmentación antociánica**		
		very small				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
14.	**(*)**	**PQ VG**	**(+) (c)**				
		Leaf: green color					
		green				Verpia	1
		yellowish green				Dorée de printemps	2
		greyish green				Celtuce, Du bon jardinier	3
15.	**(*)**	**QN VG**	**(+) (c)**				
		Leaf: intensity of green color	**Feuille: intensité de couleur verte**	**Blatt: Intensität der Grünfärbung**	**Hoja: intensidad del color verde**		
		very light	très claire	sehr hell	muy clara		1
		light	claire	hell	clara	Blonde maraîchère, Lollo Bionda	3
		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
16.		**QN VG**	**(c)**				
		Leaf: glossiness of upper side	**Feuille: brillance de la face supérieure**	**Blatt: Glanz der Oberseite**	**Hoja: brillo de la parte superior**		
		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Divina, Du bon jardinier	1
		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
17.	**(*)**	**QN VG**	**(a), (c)**				
		Leaf: thickness					
		thin	mince	dünn	delgado	Bijou, Lollo rossa, Raisa	3
		medium	moyen	mittel	medio	Curtis, Expedition	5
		thick	épais	dick	grueso	Frilett, Roxette	7

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
18.	**(*)**	**QN** **VG**	**(c)**				
		Leaf: blistering	**Feuille : cloqûre**	**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
19.		**QN** **VG/VS**	**(+)** **(c)**				
		Leaf: size of blisters					
		small				Dorée de printemps, Rodagio	3
		medium				Clarion	5
		large				Fiorella	7
20.	**(*)**	**QN** **VG/VS**	**(+)** **(a), (c)**				
		Leaf: undulation of margin					
		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
21.		**PQ** **VG**	**(+)** **(c)**				
		Leaf: type of incisions of margin					
		crenate				Gloire du Dauphiné	1
		regularly dentate				Soliflore	2
		irregularly dentate				Rodagio	3
		bidentate				Great Lakes 118	4
		tridentate				Expedition	5

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
22.		**QN VG**	**(+) (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
23.		**QN VG**	**(+) (c)**				
		Only varieties with type of incisions irregularly dentate, bi- or tridentate: Leaf: depth of secondary incisions of margin					
		shallow				Great Lakes 659	3
		medium				Expedition	5
		deep					7
24.		**QN VG**	**(+) (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
25.	**(*)**	**QN VG**	**(+) (a), (c)**				
		Leaf: venation					
		not flabellate				Verpia, Xanadu	1
		semi flabellate				Kibrille, Muraï	2
		flabellate				Locarno, Roxette	3

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
26.		**QN MS/VG**	**(b)**				
		Only varieties with degree of overlapping of upper part of leaves medium or strong: 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
27.	**(*)**	**PQ VG**	**(+) (a), (b)**				
		Only varieties with degree of overlapping of upper part of leaves medium or strong: Head: shape in longitudinal section					
		narrow elliptic				Actarus, Verte maraîchère	1
		broad elliptic				Amadeus, Sucrine	2
		circular				Verpia	3
		transverse broad elliptic				Ametist	4
28.		**QN VG**	**(b)**				
		Only varieties with degree of overlapping of upper part of leaves medium or strong: Head: density					
		loose				Nanda	3
		medium				Daguan, Delice	5
		dense				Atella, Islandia	7
		very dense				Rubette	9
29.		**QN MS/VG**	**(+) (b)**				
		Only Stem type varieties: Stem: length					
		short				Wuweijianye	3
		medium				Zipixiang	5
		long				Guasihong	7

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
30.	**QN MS/VG**	**(+) (b)**				
	Only Stem type varieties: Stem: diameter					
	small				Ailaowoju	1
	medium				Guasihong, Zipixiang	2
	large				Guasihong	3
31.	**PQ VG**	**(+) (b)**				
	Only Stem type varieties: Stem: shape in longitudinal section					
	cylindrical				Chiwoju	1
	conical				Guasihong	2
	fusiform				Zipixiang	3
32.	**PQ VG**	**(b)**				
	Only Stem type varieties: Stem: color					
	whitish green				Wuweijianye	1
	light green				Chiwoju	2
	medium green				Yangwoju	3
	greenish purple				Guasihong	4
	purplish red				Hongwosun	5
33.	**PQ VG**	**(b)**				
	Only Stem type varieties: Stem: color of flesh					
	yellowish white				Wuweijianye	1
	whitish green				Chiwoju	2
	light green				Yangwoju	3
	medium green				Guasihong	4
	dark green				Chiwosun	5

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
34.		**QN MG/VG**					
		Only varieties with degree of overlapping of upper part of leaves medium or strong: 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
35.	**(*)**	**QN MG/VG**	**(+)**				
		Time of beginning of bolting					
		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
36.		**QN VG**	**(+)**				
		Axillary sprouting					
		absent or weak				Claridia, Shotter, Valmaine, Xanadu	1
		medium				Actarus	2
		strong				Amible, Bassoon	3
37.		**QN VG**	**(+)**				
		Bolting 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, Verdetrix	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
38.	**(*)**	**QL** **VG**	**(+)**				
		Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 16					
		absent				Green Towers	1
		present				Argelès	9
39.		**QL** **VG**					
		Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 17					
		absent				Green Towers	1
		present				Argelès	9
40.		**QL** **VG**					
		Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 20					
		absent				Green Towers	1
		present				FrRsal-1	9
41.		**QL** **VG**					
		Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 21					
		absent				Green Towers	1
		present				Argelès, Colorado	9
42.		**QL** **VG**					
		Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 22					
		absent				Green Towers	1
		present				FrRsal-1	9
43.		**QL** **VG**					
		Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 23					
		absent				Green Towers	1
		present				Colorado	9

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
44.	**QL** **VG**					
	Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 24					
	absent				Argelès, Colorado	1
	present				Dandie, NunDm15, UCDm14	9
45.	**QL** **VG**					
	Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 25					
	absent				Colorado	1
	present				Argelès	9
46.	**QL** **VG**					
	Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 26					
	absent				Colorado	1
	present				Balesta, Bedford	9
47.	**QL** **VG**					
	Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 27					
	absent				Balesta, Colorado	1
	present				FrRsal-1	9
48.	**QL** **VG**					
	Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 29					
	absent				Argelès	1
	present				Balesta	9
49.	**QL** **VG**					
	Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 30					
	absent				Argelès, Colorado	1
	present				Balesta	9

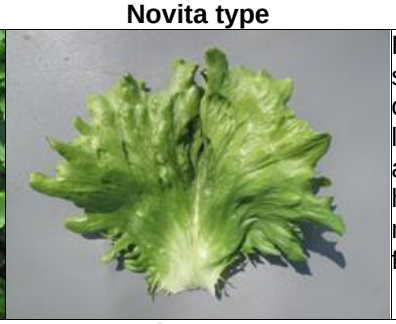
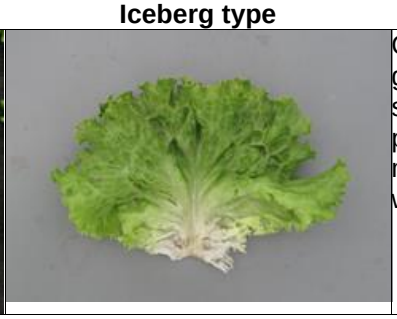
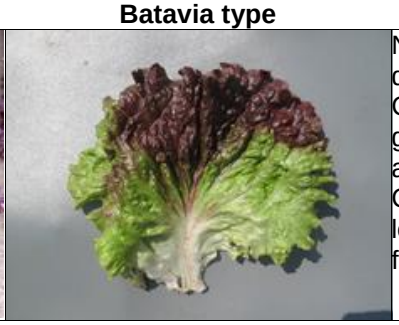
	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
50.	**QL VG**					
	Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 31					
	absent				Colorado, RYZ910457	1
	present				Argelès, Balesta	9
51.	**QL VG**	**(+)**				
	Resistance to *Lettuce mosaic virus* (LMV) pathotype II					
	absent				Bijou, Hilde II, Sprinter, Sucrine	1
	present				Capitan, Corsica	9
52.	**QL VG**	**(+)**				
	Resistance to *Nasonovia ribisnigri* biotype Nr: 0					
	absent				Abel, Green Towers, Nadine	1
	present				Barcelona, Bedford, Dynamite, Silvinas	9
53.	**QN MS/VG**	**(+)**				
	Resistance to *Fusarium oxysporum* f.sp. *lactucae* race 1					
	susceptible				Cobham Green, Patriot	1
	moderately resistant				Affic, Fuzila, Natexis	2
	highly resistant				Costa Rica No. 4, Romasol	3

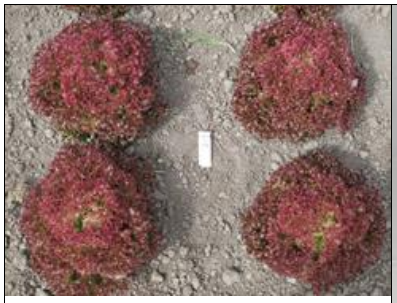
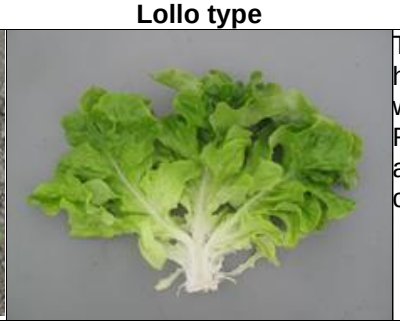
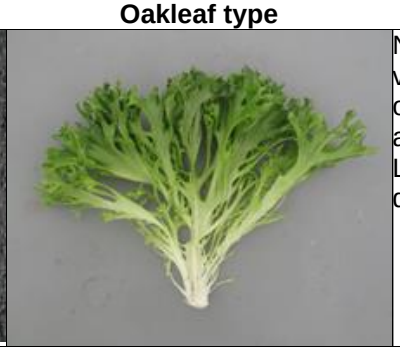
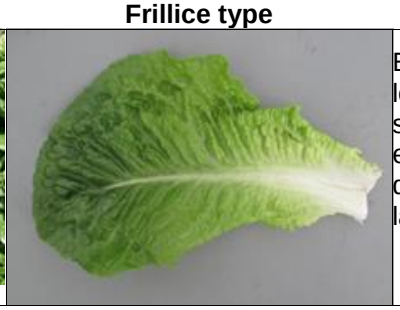
8. Explanations on the Table of Characteristics

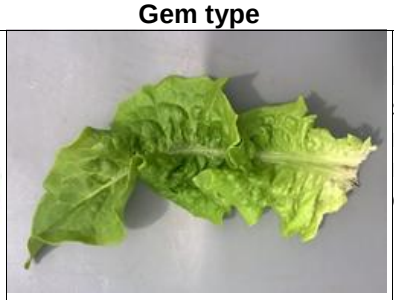
8.1 Explanations covering several characteristics

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

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

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		
		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.
Frisée d'Amérique 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.
Lollo 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.
Oakleaf type		
		Non-heading; thin, medium to very strong divided leaves. Tip of divisons can be undulated and incised. Plant may look as a Lollo type, but leaves are always divided.
Multi-divided type		
		Non-heading; thick, crispy leaves, sometimes weakly divided. Clearly incised leaf margin.
Frillice 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.
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 condtions; leaves are mainly tough and have a clear midrib. Leaves and/or stem are consumed.
Stem type		

(b) Plant, head and stem: Observations should be made at harvest maturity. For varieties with degree of overlapping absent or weak observations should be made just before deterioration and before bolting.

(c) Leaf: For varieties with degree of overlapping medium or strong observations should be made on the largest outer leaves, at harvest maturity. For varieties with degree of overlapping 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 degree of overlapping of upper part of leaves absent or weak: Plant: number of leaves

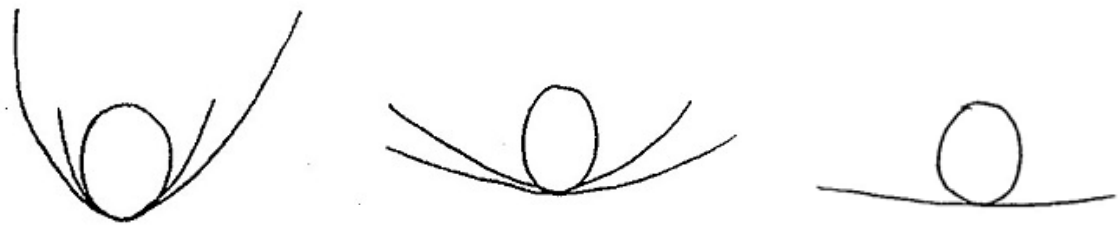
Observations should be made on the whole plant.

1
few

3
medium

5
many

Ad. 5: Leaf: attitude



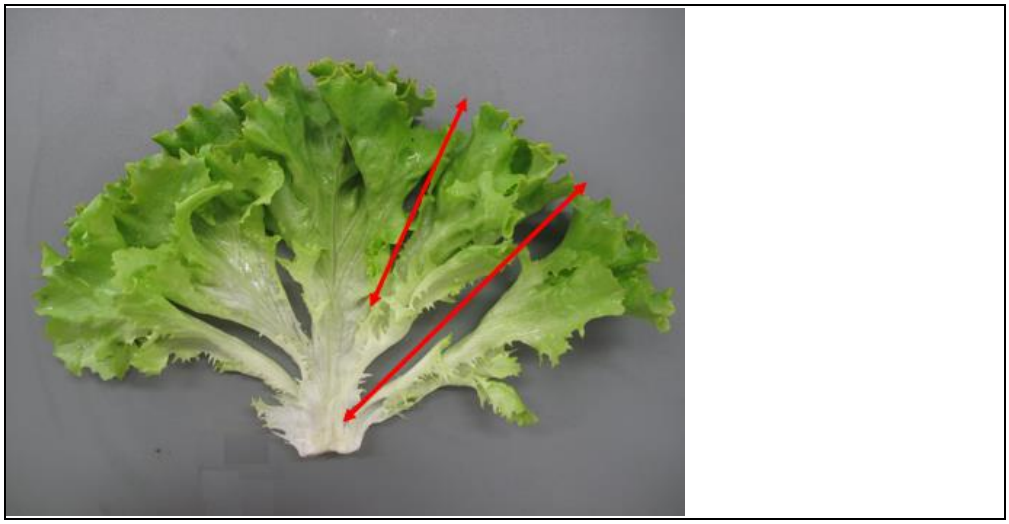
1
erect

3
semi-erect

5
horizontal

Ad. 6: Leaf: number of divisions

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

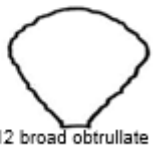
Ad. 7: Only Oakleaf type varieties: Leaf: width of lobes

3
narrow

5
medium

7
broad

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

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

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

1
obtuse

2
rounded

3
obcordate

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

1
concave

3
flat

5
convex

Ad. 11: Leaf: 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 Trotzkopf, 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	Iride

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 Trotzkopf, 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	Iride

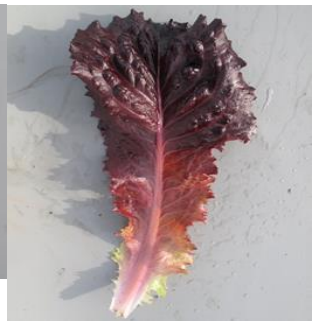
Ad. 13: Leaf: area covered by anthocyanin coloration

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: green color

Observations to be made only for green varieties and for two-colored varieties with 'Leaf: area covered by anthocyanin coloration' less than medium.

Intensity of green color (Ch. 15)	Green color (Ch. 14)		
	1 green	2 yellowish green	3 greyish green
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, Verdetrix		

Ad. 15: Leaf: intensity of green color

Observations should be made only for green varieties and for two-colored varieties with an area covered with anthocyanin less than medium.

Intensity of green color (Ch. 15)	Green color (Ch. 14)		
	1 green	2 yellowish green	3 greyish green
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, Verdetrix		

Ad. 19: Leaf: size of blisters

Observations should be made on the whole leaf.

3	5	7
small	medium	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.

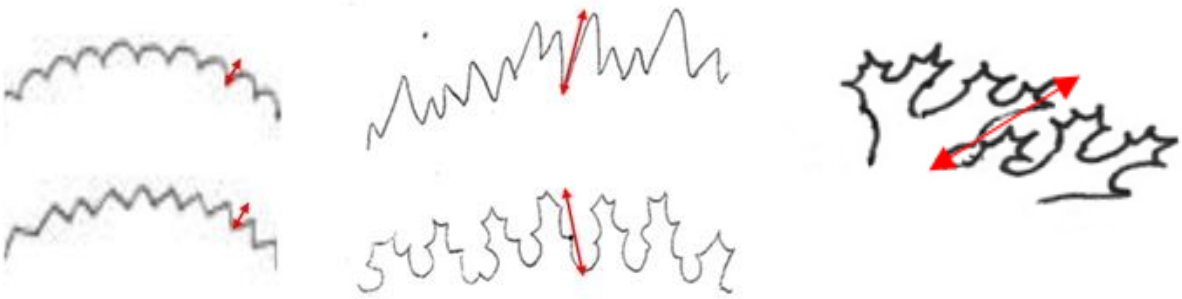
Ad. 21: Leaf: type of incisions of margin

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

1	2	3	4	5
crenate	regularly dentate	irregularly dentate	bidentate	tridentate

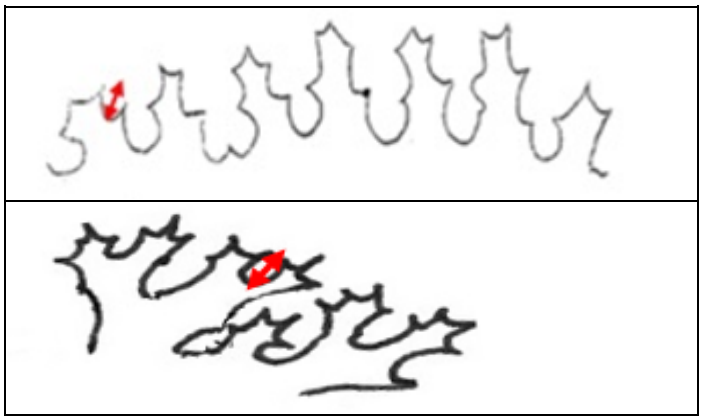
Ad. 22: Leaf: depth of incisions of margin

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



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

Observations should be made on secondary incisions of the margin at distal half. In case of tridentate incisions do not observe tertiary incisions of the margin (the most shallow ones).



Ad. 24: Leaf: density of incisions of margin

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

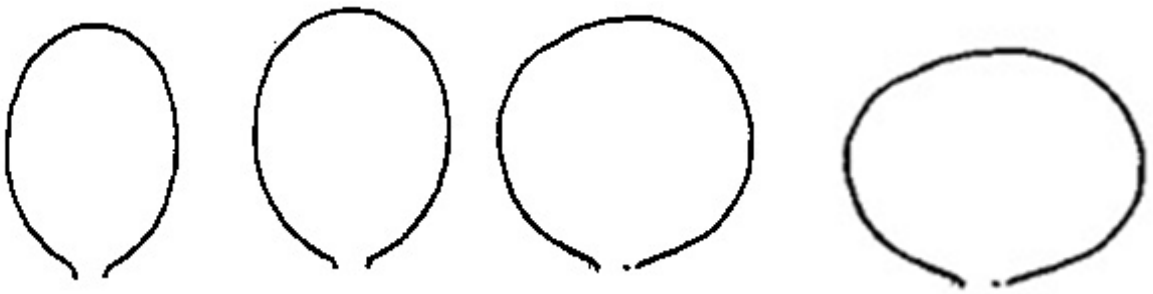
Ad. 25: Leaf: venation

1
not flabellate

2
semi flabellate

3
flabellate

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



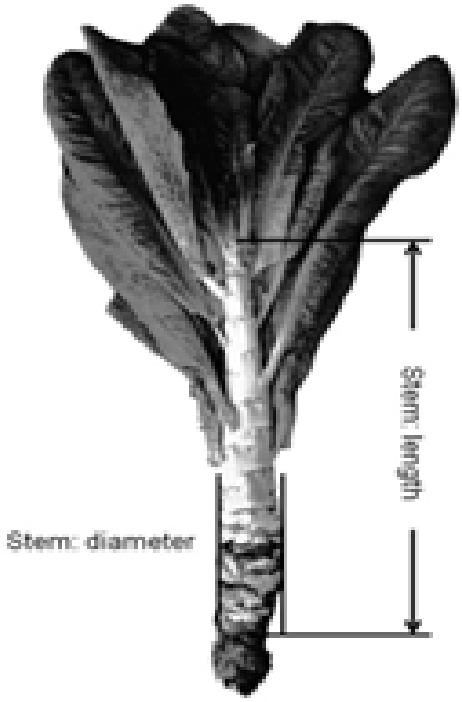
1
narrow elliptic

2
broad elliptic

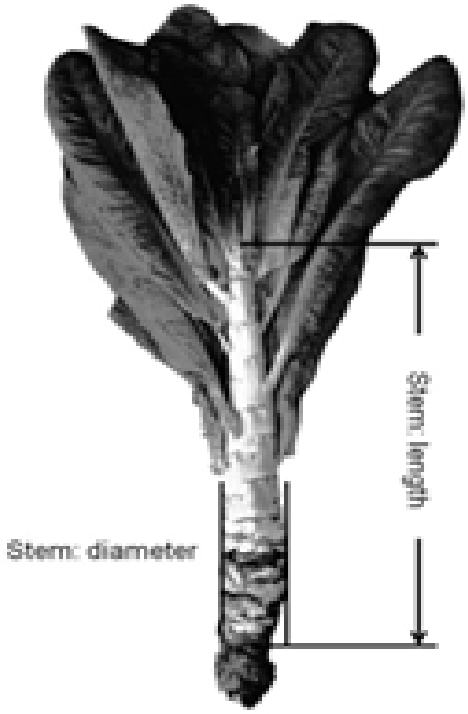
3
circular

4
transverse broad elliptic

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

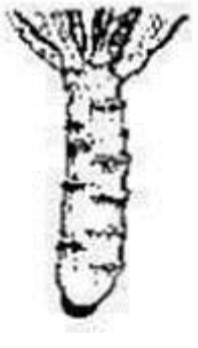


Ad. 30: Only Stem type varieties: Stem: diameter

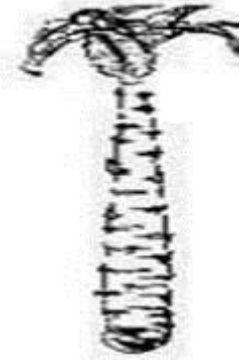


Observations should be made on the broadest part of the stem.

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

Observations should be made at the start of bolting.

Ad. 37: Bolting 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 with strong degree of overlapping: 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: Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 16

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: 16,17, 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 plantlets	
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 3.10^4-1.10^5	
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 observation) 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 http://www.worldseed.org/our-work/plant-health/other-initiatives/ibeb/ . The table for isolates mentioned in this guideline and illustrations for the observation scale are given.

Isolates / Differentials	GreenTowers	Dandie	R4T57D	UCDm14	NunDm15	CGDm16	Colorado	FrRsal-1	Argelès	RYZ 2164	RYZ910457	Bedford	Balesta	Bartoli	Design
Bl: 16	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-
Bl: 17	+	+	-	+	+	-	+	+	-	-	-	(+)	-	-	-
Bl: 20	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-
Bl: 21	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-
Bl: 22	+	-	+	+	+	-	+	-	-	-	-	-	+	-	-
Bl: 23	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-
Bl: 24	+	-	+	-	-	+	+	-	+	-	-	-	-	-	(-)
Bl: 25	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-
Bl: 26	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-
Bl: 27	+	+	+	+	+	-	+	-	+	+	-	(-)	+	-	-
Bl: 29	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-
Bl: 30	+	-	+	+	+	-	+	-	+	+	-	-	-	-	+
Bl: 31	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+

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

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Ad. 51: Resistance to *Lettuce mosaic virus* (LMV) pathotype II

1. Pathogen	Lettuce mosaic virus
2. Quarantine status	No
3. Host species	Lettuce - *Lactuca sativa* L.
4. Source of inoculum	GEVES[1] (FR) or Naktuinbouw[2] (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	LMV buffer (e.g. 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)
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 R or S 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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Ad. 52: Resistance to *Nasonovia ribisnigri* biotype Nr: 0

1. Pathogen	*Nasonovia ribisnigri*
2. Quarantine status	no
3. Host species	lettuce - *Lactuca sativa* L.
4. Source of inoculum	Naktuinbouw[1] (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 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	include control varieties
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
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

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Ad. 53: Resistance to *Fusarium oxysporum* f.sp. *lactucae* 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[1] (JP), CRA-SCS[2] (IT), Naktuinbouw[3] (NL), GEVES[4] (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 ºC (day) / 20 ºC (night)
9.7 Light	under natural day length

10. Inoculation	
10.1 Preparation inoculum	• 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	• inoculation by sowing on contaminated soil: soil : culture = 20 : 1 • inoculation by soaking seedlings: spores are harvested and adjusted to 10^6 to 10^7 sp/ml
10.3 Plant stage at inoculation	• 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	• inoculation by sowing on contaminated soil:
	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	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:

	Application date: (not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE
to be completed in connection with an application for plant breeders' rights

1. Subject of the Technical Questionnaire

1.1	Botanical name	*Lactuca sativa* L.
1.2	Common name	Lettuce

2. Applicant

Name	
Address	
Telephone No.	
Fax No.	
E-mail address	
Breeder (if different from applicant)	

3. Proposed denomination and breeder's reference

Proposed denomination (if available)	
Breeder's reference	

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

#4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]

(b) partially known cross [ ]

(c) unknown cross [ ]

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)

Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

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)

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

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	**Seed: color**		
(1)			
	white	Verpia	1 [ ]
	yellow	Durango	2 [ ]
	black	Kagraner Sommer 2	3 [ ]
5.2	**Leaf: anthocyanin coloration**		
(11)			
	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	**Leaf: intensity of green color**		
(15)			
	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 [ ]

	Characteristics	Example Varieties	Note
5.4	**Time of beginning of bolting**		
(35)			
	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 [ ]
5.5	**Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 16**		
(38)			
	absent	Green Towers	1 [ ]
	present	Argelès	9 [ ]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

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:

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

#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

Type (see 5.3 and 8.1 for explanations):

Type	**Example varieties**	
Butterhead type	Clarion, Maikönig, Sartre	[ ]
Novita type	Norvick	[ ]
Iceberg type	Great Lakes 659, Roxette, Saladin, Vanguard 75	[ ]
Batavia type	Aquarel, Curtis, Funnice, Felucca, Grand Rapids, Masaida, Visyon	[ ]
Frisée d'Amérique type	Bijou, Blonde à couper améliorée	[ ]
Lollo type	Lollo rossa, Revolution	[ ]
Oakleaf type	Catalogna, Kipling, Muraï, Salad Bowl	[ ]
Multi-divided type	Curletta, Duplex, Jadigon, Rodagio	[ ]
Frillice type	Frilett	[ ]
Cos type	Actarus, Blonde maraîchère, Pinokkio	[ ]
Gem type	Craquerelle du Midi, Sucrine, Xanadu	[ ]
Stem type	Celtuce, Guasihong	[ ]

Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

Resistances:

(39) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 17

not tested		0 [ ]
absent	Green Towers	1 [ ]
present	Argelès	9 [ ]

(40) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 20

not tested		0 [ ]
absent	Green Towers	1 [ ]
present	FrRsal-1	9 [ ]

(41) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 21

not tested		0 [ ]
absent	Green Towers	1 [ ]
present	Argelès, Colorado	9 [ ]

(42) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 22

not tested		0 [ ]
absent	Green Towers	1 [ ]
present	FrRsal-1	9 [ ]

(43) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 23

not tested		0 [ ]
absent	Green Towers	1 [ ]
present	Colorado	9 [ ]

(44) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 24

not tested		0 [ ]
absent	Argelès, Colorado	1 [ ]
present	Dandie, NunDm15, UCDm14	9 [ ]

(45) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 25

not tested		0 [ ]
absent	Colorado	1 [ ]
present	Argelès	9 [ ]

(46) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 26

not tested		0 [ ]
absent	Colorado	1 [ ]
present	Balesta, Bedford	9 [ ]

(47) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 27

not tested		0 [ ]
absent	Balesta, Colorado	1 [ ]
present	FrRsal-1	9 [ ]

(48) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 29

not tested		0 [ ]
absent	Argelès	1 [ ]
present	Balesta	9 [ ]

(49) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 30

not tested		0 [ ]
absent	Argelès, Colorado	1 [ ]
present	Balesta	9 [ ]

(50) Resistance to downy mildew (*Bremia lactucae*) isolate Bl: 31

not tested		0 [ ]
absent	Colorado, RYZ910457	1 [ ]
present	Argelès, Balesta	9 [ ]

Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

(51)	Resistance to *Lettuce mosaic virus* (LMV) pathotype II		
	not tested		0 [ ]
	absent	Bijou, Hilde II, Sprinter, Sucrine	1 [ ]
	present	Capitan, Corsica	9 [ ]
(52)	Resistance to *Nasonovia ribisnigri* biotype Nr: 0		
	not tested		0 [ ]
	absent	Abel, Green Towers, Nadine	1 [ ]
	present	Barcelona, Bedford, Dynamite, Silvinas	9 [ ]

Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

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.

9. Information on plant material to be examined or submitted for examination

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

9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

(a)	Microorganisms (e.g. virus, bacteria, phytoplasma)	Yes [ ]	No [ ]
(b)	Chemical treatment (e.g. growth retardant, pesticide)	Yes [ ]	No [ ]
(c)	Tissue culture	Yes [ ]	No [ ]
(d)	Other factors	Yes [ ]	No [ ]

Please provide details for where you have indicated “yes”.

.....

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant’s name

Signature Date