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TG/13/11(proj.1)
ORIGINAL: English
DATE: 2014-05-14

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 experts from The Netherlands

to be considered by the

Technical Working Party for Vegetables
at its forty-eighth session, to be held in Paestum, Italy, from June 23 to 27, 2014

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.

Further guidance is provided in documents TGP/9 “Examining Distinctness” and TGP/8 “Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability”.

4.1.2 Consistent Differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear Differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these 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 In the first place, the collection should be divided according to growth types and subtypes in Table 1.

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

5.4 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 30)
(d) Resistance to downy mildew (*Bremia lactucae*): Isolate Bl: 16 (characteristic 32.1)

5.5 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)-(b)	See Explanations on the Table of Characteristics in Chapter 8.2.	
(+)	See Explanations on the Table of Characteristics in Chapter 8.3.	

Table 1.

Plant: growth type	**Plant: growth sub-type**	**Example varieties**	**Plant: head formation (3)**	**Only cutting lettuce varieties: Plant: number of leaves (4)**	**Leaf: division (6)**	**Leaf: thickness (18)**	**Leaf: undulation of margin (21)**	**Leaf: venation (24)**	**Only varieties with closed head: Head: shape in longitudinal section (28)**
Butterhead lettuce	-	Clarion, Maikönig	closed head	-	entire	thin to thick	absent to weak	not flabellate	circular or transverse broad elliptic
Crisp lettuce	Iceberg	Great Lakes 659, Roxette, Saladin, Vanguard 75	closed head	-	entire	thick	absent to medium	flabellate	circular or transverse broad elliptic
	heading Batavia	Curtis, Masaida, Visyon	closed head	-	entire	medium to thick	weak to strong	flabellate	broad elliptic, circular or transverse broad elliptic
	open heading Batavia	Aquarel, Funnice	open head	-	entire	medium to thick	medium to very strong	flabellate	-
Cos lettuce	-	Actarus, Blonde maraîchère, Pinokkio	open head or closed head	-	entire	medium to thick	absent to weak	not flabellate	narrow elliptic
Grasse lettuce	-	Craquerelle du Midi, Sucrine, Xanadu	open head or closed head	-	entire	medium to thick	absent to weak	not flabellate	broad elliptic, circular or transverse broad elliptic
Cutting lettuce	Frisée d'Amérique	Bijou, Faradia, Grand Rapids	no head	small or medium	entire	thin	absent to very strong	flabellate or not flabellate or semi	-
	Oakleaf	Catalogna, Kipling, Muraï, Salad Bowl	no head	small or medium	divided	thin	absent to weak	flabellate or not flabellate or semi	-
	Frillice	Frilett	no head	small or medium	entire	thick	weak to strong	flabellate	-
	Lollo	Lollo rossa, Revolution	no head	small or medium	entire	thin	strong to very strong	flabellate	-
	Divided	Curletta, Duplex, Jadigon, Rodagio	no head	small or medium	divided	thin	weak to very strong	flabellate	-
	Multileaf	Felluca, Sartre, Xeres	no head	large	entire or divided	thin to medium	absent to very strong	flabellate or not flabellate or semi	-
Novita lettuce	-	Norvick	open head	-	entire	thin to medium	very weak to medium	flabellate	circular or transverse broad elliptic
Stem lettuce	-	Celtuce	no head	-	entire	thin to medium	absent to weak	not flabellate	-

Section 8.1 provides illustrations for the growth (sub-)types.

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

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1. **(*)**	**VG**	**Seed: color**					
QL		white				Verpia	1
		yellow				Durango	2
		black				Kagraner Sommer 2	3
~~**2.**~~ ~~**(*)**~~ ~~**(+)**~~	~~**VG**~~	~~**Seedling: anthocyanin coloration**~~					
~~**QL**~~		~~absent~~				~~Verpia~~	~~1~~
		~~present~~				~~Pirat~~	~~9~~
~~**3.**~~	~~**VG**~~	~~**Seedling: size of cotyledon (fully developed)**~~					
~~**QN**~~		~~small~~				~~Romance~~	~~3~~
		~~medium~~				~~Expresse~~	~~5~~
		~~large~~				~~Verpia~~	~~7~~
~~**4.**~~	~~**VG**~~	~~**Seedling: shape of cotyledon**~~					
~~**QN**~~		~~narrow elliptic~~				~~Calmar~~	~~3~~
		~~medium elliptic~~				~~Frisette~~	~~5~~
		~~broad elliptic~~				~~Fiorella, Sunrise~~	~~7~~
~~**5.**~~	~~**VG**~~	~~**Leaf: attitude at 10-12 leaf stage**~~					
~~**QN**~~		~~erect~~				~~Baby Star, Romance~~	~~1~~
		~~semi-erect~~				~~Great Lakes 118, Soraya~~	~~3~~
		~~prostrate~~				~~Unicum, Vanguard 75~~	~~5~~
2. **(old 7)** **(*)**	**VG**	**Plant: diameter**					
QN	**(a)**	very small				~~Pavane,~~ Tom Thumb	1
		small				~~Bastion,~~ Gotte à graine blanche	3
		medium				Clarion, Verpia	5
		large				Great Lakes 659~~, Musette~~	7
		very large				El Toro~~, Yuma~~	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
3. **(old 8)** **(*)** **(+)**	**VG**	**Plant: head formation**					
PQ	**(a)**	no head				Blonde à couper améliorée, Lollo rossa, Redair	1
		open head				Actarus, Aquarel ~~Manfred, Monet~~	2
		closed head				Clarion, Roxette, ~~Kelvin,~~ ~~Sunrise~~	3
4. **(+)**	**VG/ MS**	**Only cutting lettuce varieties: Plant: number of leaves**					
QN	**(a)**	small				Lollo rossa	1
		medium				Salad Bowl	2
		large				Felluca, Sartre, Xeres	3
5. **(old 15)** **(+)**	**VG**	**Leaf: attitude**					
QN	**(b)**	erect				Feria, Pinokkio~~, Riva~~	1
		semi-erect				~~Amelia~~, Faradia, Sartre, ~~Toronto~~	3
		horizontal				~~Chambery,~~ Divina	5
6. **(+)**	**VG**	**Leaf: division**					
QL	**(b)**	entire				Fiorella, Lollo rossa, ~~Sunrise~~	1
		~~lobed~~				~~A couper à feuille de chêne blonde à graine noire, Salad Bowl~~	~~2~~
		divided				Jadigon, Kipling, Lagon, ~~Monet~~	2

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
7. **(+)**	**VG**	**Only varieties with divided leaves: Leaf: number of divisions**					
QN	**(b)**	very small					1
		small				Curletta, Rodagio	3
		medium				Ezabel, Jadigon	5
		large				Expedition, Multired 54	7
		very large				Excite, Ezfrill, Telex	9
8. **(+)**	**VG**	**Only oakleaf sub-types: Leaf: width of lobes**					
QN	**(b)**	narrow				Kibrille, Rougini	3
		medium				Bandolin, Ribaï	5
		broad				Horix, Starix, Vizir	7
9. **(old 16)** **(*)** **(+)**	**VG**	**Only varieties with entire leaves: Leaf: shape**					
PQ	**(b)**	narrow elliptic				Riva, Verte maraîchère	1
		medium elliptic				Angela, Xanadu	2
		broad elliptic				Amadeus, Amelia	3
		circular				Elsa, Sunrise, Verpia	4
		transverse broad elliptic				Commodore, Fiorella	5
		transverse narrow elliptic				Elvira, Madison, Stylist	6
		obovate				Raisa, Toronto	7
		broad obtrullate				Delicato, Monet	8
		triangular				Amboni, Deer Tongue	9
~~**17.**~~	~~**VG**~~	~~**Leaf: shape of tip**~~					
~~**PQ**~~	~~**(b)**~~	~~acute~~				~~Celtuce, Deer Tongue, Karola, Tempra~~	~~1~~
		~~obtuse~~				~~Chicon des Charentes, Grise maraîchère~~	~~2~~
		~~rounded~~				~~Blonde Maraîchère, Maserati~~	~~3~~

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
10. **(+)**	**VG**	**Only varieties with entire leaves: Leaf: cross section**					
QN	**(b)**	convex				Tiago	1
		flat				Clarion, Lollo rossa	3
		concave				Sunstar	5
11. **(old 20)** **(*)** **(+)**	**VG**	**Leaf: anthocyanin coloration**					
QL	**(b)**	absent				Clarion, ~~Fiorella, Sunrise~~	1
		present				~~Commodore~~, Lollo rossa, ~~Pirat~~	9
12. **(+)**	**VG**	**Leaf: area covered with anthocyanin coloration**					
QN	**(b)**	very small				Steirer Krauthauptel	1
		small				Diablo	3
		medium				Luana	5
		large				Merveille des quatre saisons	7
		very large				Bijou, Revolution	9
13. **(+)**	**VG**	**Leaf: hue of anthocyanin coloration**					
PQ	**(b)**	reddish				Lollo rossa	1
		brownish				Brauner Trotzkopf, Luana	2
		purplish				Faradia, Iride	3
14. **(old 21)** **(*)** **(+)**	**VG**	**Leaf: intensity of anthocyanin coloration**					
QN	**(b)**	very weak				~~Chicon de Charentes, Muranta, Rumina~~	1
		weak				Du bon jardinier	3
		medium				Lollo rossa, Luana, ~~Trocadéro à graine noire~~	5
		strong				~~Amandine,~~ Merveille des quatre saisons	7
		very strong				~~Little Leprechaun,~~ Iride, Revolution	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
~~**22.**~~	~~**VG**~~	~~**Leaf: distribution of anthocyanin**~~					
~~**QL**~~	~~**(b)**~~	~~localised~~				~~Muranta, Rumina~~	~~1~~
		~~entire~~				~~Delicato, Liberty~~	~~2~~
~~**23.**~~	~~**VG**~~	~~**Leaf: kind of anthocyanin distribution**~~					
~~**QL**~~	~~**(b)**~~	~~diffused only~~				~~Amandine, Pirat, Sanguine~~	~~1~~
		~~in spots only~~				~~Passion blonde à graine blanche, Unicum~~	~~2~~
		~~diffused and in spots~~				~~Lovina, Rougette du Midi~~	~~3~~
15. **(old 18)** **(*)** **(+)**	**VG**	**Leaf: hue of green color**					
PQ	**(b)**	absent				~~Donatello,~~ Verpia	1
		yellowish				Dorée de printemps	2
		greyish				Celtuce, Du bon jardinier	3
16. **(old 19)** **(*)** **(+)**	**VG**	**Leaf: intensity of green color**					
QN	**(b)**	very light					1
		light				Blonde maraîchère, Lollo	3
		medium				Aquarel, Clarion	5
		dark				Expedition, Verpia	7
		very dark				Pascal, Verdetrix	9
17. **(old 24)**	**VG**	**Leaf: glossiness of upper side**					
QN	**(b)**	absent or very weak				Divina, Du bon jardinier	1
		weak				Duplex, ~~Elsa,~~ Fiorella, Sartre	3
		medium				~~Feria~~, Funnice, ~~Sunrise~~	5
		strong				~~Ibis~~, Noisette, Redair	7
		very strong				Bijou	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
18. (old 14)	**VG**	**Leaf: thickness**					
QN	**(b)**	thin				Bijou, Lollo rossa, Raisa, ~~Royal Red~~	3
		medium				~~Dustin~~, Curtis, Expedition, ~~Sunrise~~	5
		thick				Frilett, ~~Frisée de Beauregard,~~ Roxette	7
19. (old 25) (*)	**VG**	**Leaf: blistering**					
QN	**(b)**	absent or very weak				~~Donia, Frillblond,~~ Duplex, Sartre	1
		weak				Fiorella, ~~Minas~~	3
		medium				Commodore, Rodagio	5
		strong				Blonde de Paris, ~~Smile,~~ Xanadu	7
		very strong				Blonde de Doulon, Iride, Karioka	9
20. (old 26)	**VG**	**Leaf: size of blisters**					
QN	**(b)**	small				Dorée de printemps, Faradia, Rodagio	3
		medium				Visyon, ~~Dustin, Sunrise~~	5
		large				Fiorella, ~~Massilia~~	7
21. (old 27)	**VG**	**Leaf: undulation of margin**					
QN	**(b)**	absent or very weak				~~Dustin, Manfred,~~ Tiago	1
		weak				Commodore~~, Sunrise~~	3
		medium				Noisette, Pentared	5
		strong				Calmar, Invicta	7
		very strong				Lollo rossa~~, Madison~~	9
~~**28.**~~	~~**VG**~~	~~**Leaf blade: incisions of margin on apical part**~~					
~~**QL**~~	~~**(b)**~~	~~absent~~				~~Verpia~~	~~1~~
		~~present~~				~~Calmar, Gloire du Dauphiné, Unicum~~	~~9~~

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
22. **(old 28 and 29)** **(+)**	**VG**	**Leaf: depth of incisions on margin of apical part**					
QN	**(b)**	absent or very shallow				Actarus, Clarion, Tiago	1
		shallow				Pentared, Unicum	3
		medium				Crispino, ~~Ithaca Great Lakes~~	5
		deep				Expedition, ~~Lagon, Monet~~	7
		very deep					9
23. **(old 30)** **(+)**	**VG**	**Leaf: density of incisions on margin of apical part**					
QN	**(b)**	very sparse					1
		sparse				Maravilla de Verano	3
		medium				Calmar~~, De Pierre Benite~~	5
		dense				Grand Rapids~~, Ithaca Great Lakes~~	7
		very dense				Locarno~~, Madison~~	9
~~**31.**~~	~~**VG**~~	~~**Varieties with shallow incisions on margin on apical part only: Leaf blade: type of incisions on apical part**~~					
~~**QL**~~	~~**(b)**~~	~~sinuate~~				~~Gloire du Dauphiné~~	~~1~~
		~~dentate~~				~~Calmar~~	~~2~~
24. **(old 32)** **(+)**	**VG**	**Leaf: venation**					
QN	**(b)**	not flabellate				~~Donatella,~~ Verpia, Xanadu	1
		semi flabellate				Kibrille, Muraï	2
		flabellate				~~Gloire du Dauphiné,~~ Locarno, ~~Monet,~~ Roxette	3
25. **(+)**	**VG**	**Only cutting lettuce varieties: Heart: density**					
QN	**(a)**	loose				Salad Bowl	3
		medium				Curletta, Kiprien	5
		dense				Livorno, Verdetrix	7

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
26. (old 9) (+)	**VG**	**Only varieties with closed head: Head: degree of overlapping of upper part of leaves**					
QN	**(a)**	very weak				~~Colorado~~, Femke	1
		weak				~~Danilla, Novita~~, Curtis	3
		medium				Augusta, Fiorella	5
		strong				~~Master, Minas,~~ Kanaria	7
		very strong				~~Kelvin~~, Roxette, Vanguard 75	9
~~**10.**~~	~~**VG**~~	~~**Head: density**~~					
~~**QN**~~	~~**(a)**~~	~~loose~~				~~Nanda~~	~~3~~
		~~medium~~				~~Blonde maraîchère~~	~~5~~
		~~dense~~				~~Hilde II~~	~~7~~
27. (old 11)	**VG/ MS**	**Only varieties with closed head: Head: size**					
QN	**(a)**	very small				Tom Thumb	1
		small				~~Bastion, Gotte à graine blanche,~~ Xanadu	3
		medium				Aquarel, Fiorella, Soraya	5
		large				Great Lakes 659~~, Musette~~	7
		very large				Blonde maraîchère	9
~~**12.**~~	~~**VG**~~	~~**Butterhead type varieties in glasshouse only: Head: closing of base**~~					
~~**QN**~~	~~**(a)**~~	~~weak~~				~~Passe Partout~~	~~3~~
		~~medium~~				~~Carmelita~~	~~5~~
		~~strong~~				~~Dustin, Manfred~~	~~7~~

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
28. (old 13) (*) (+)	**VG**	**Only varieties with closed head: Head: shape in longitudinal section**					
PQ	**(a)**	narrow elliptic				Actarus, Verte maraîchère	1
		broad elliptic				Amadeus, Aquarel, Sucrine	2
		circular				~~Passe Partout,~~ Verpia	3
		transverse broad elliptic				Ametist, Frisady	4
~~**30.**~~	~~**VG**~~	~~**Axillary sprouting**~~					
~~**QN**~~		~~absent or very weak~~				~~Valmaine, Xanadu~~	~~1~~
		~~weak~~				~~Claridia, Shotter~~	~~3~~
		~~medium~~				~~Actarus~~	~~5~~
		~~strong~~				~~Amible, Bassoon~~	~~7~~
		~~very strong~~					~~9~~
29. (old 34)	**VG/ MG**	**Only varieties with closed head: Time of harvest maturity**					
QN		very early				~~Blonde à couper améliorée,~~ Gotte jaune d'or	1
		early				Attractie, Pantlika	3
		medium				Clarion~~, Newton~~	5
		late				Blonde maraîchère, Calmar	7
		very late				El Toro, Pinokkio	9
30. (old 35) (*) (+)	**VG/ MG**	**Time of beginning of bolting under long day conditions**					
QN		very early				Blonde à couper améliorée	1
		early				Gotte à graine blanche	3
		medium				~~Carélia,~~ Pantlika	5
		late				Hilde II	7
		very late				Erika, ~~Kinemontepas, Rex,~~ Roxette	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
~~**36.**~~	~~**VG/ MG**~~	~~**Plant: height (flowering plant)**~~					
~~**QN**~~		~~short~~				~~Gotte à graine blanche~~	~~3~~
		~~medium~~				~~Samourai~~	~~5~~
		~~tall~~				~~Danilla, Hilde II~~	~~7~~
~~**37.**~~	~~**VG**~~	~~**Plant: fasciation (at flowering stage)**~~					
~~**QL**~~		~~absent~~				~~Calmar, Romance~~	~~1~~
		~~present~~				~~Gotte jaune d'or~~	~~9~~
31. **(old 37 and 38)**	**VG**	**Plant: fasciation**					
(+)							
QN		absent or very weak				Aquarel, Gotte à graine blanche	1
		weak				Verte maraîchère	2
		medium				Amadeus	3
		strong				~~Gotte jaune d'or,~~ Rougini	4
		very strong				~~Chicon des Charentes,~~ Sartre, Verdetrix	5
32. **(old 39)**	**VG**	**Resistance to downy mildew (*Bremia lactucae*)**					
(+)							
QL							
~~**39.1**~~		~~**Isolate Bl: 2**~~					
		~~absent~~				~~Green Towers~~	~~1~~
		~~present~~				~~Ninja~~	~~9~~
~~**39.2**~~		~~**Isolate Bl: 5**~~					
		~~absent~~				~~Green Towers~~	~~1~~
		~~present~~				~~Sabine~~	~~9~~
~~**39.3**~~		~~**Isolate Bl: 7**~~					
		~~absent~~				~~Green Towers~~	~~1~~
		~~present~~				~~Valmaine~~	~~9~~

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
~~**39.4**~~		~~**Isolate Bl: 12**~~					
		~~absent~~				~~Green Towers~~	~~1~~
		~~present~~				~~Dandie, UCDM2~~	~~9~~
~~**39.5**~~		~~**Isolate Bl: 14**~~					
		~~absent~~				~~Green Towers~~	~~1~~
		~~present~~				~~Colorado, Ninja~~	~~9~~
~~**39.6**~~		~~**Isolate Bl: 15**~~					
		~~absent~~				~~Green Towers~~	~~1~~
		~~present~~				~~Colorado, Sabine~~	~~9~~
32.1 **(*)**		**Isolate Bl: 16**					
		absent				Green Towers	1
		present				Argelès, Ninja	9
32.2		**Isolate Bl: 17**					
		absent				Green Towers	1
		present				Argelès, Ninja	9
~~**39.9**~~	~~**(c)**~~	~~**Isolate Bl: 18**~~					
		~~absent~~				~~Green Towers~~	~~1~~
		~~present~~				~~Argelès, Ninja~~	~~9~~
32.3		**Isolate Bl: 20**					
		absent				Green Towers	1
		present				Argelès, Ninja	9
32.4		**Isolate Bl: 21**					
		absent				Green Towers	1
		present				Argelès, Colorado	9
32.5		**Isolate Bl: 22**					
		absent				Green Towers	1
		present				Discovery, Ninja	9
32.6		**Isolate Bl: 23**					
		absent				Green Towers	1
		present				Colorado, Discovery, Ninja	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
32.7		**Isolate Bl: 24**					
		absent				Argelès, Colorado	1
		present				Dandie, NunDm15, UC DM14	9
32.8		**Isolate Bl: 25**					
		absent				Colorado, Discovery	1
		present				Argelès, Ninja	9
32.9		**Isolate Bl: 26**					
		absent				Colorado, Discovery	1
		present				Balesta, Bedford	9
32.10		**Isolate Bl: 27**					
		absent				Balesta, Colorado	1
		present				~~Bedford,~~ Discovery, Ninja	9
32.11		**Isolate Bl: 28**					
		absent				Argelès, Colorado	1
		present				Bedford, Discovery	9
32.12		**Isolate Bl: 29**					
		absent				Argelès, Discovery	1
		present				Balesta, Ninja	9
32.13		**Isolate Bl: 30**					
		absent				Argelès, Colorado	1
		present				Balesta, Ninja	9
32.14		**Isolate Bl: 31**					
		absent				Colorado, RYZ910457	1
		present				Argelès, Balesta	9
33. (old 40)	**VG**	**Resistance to lettuce mosaic virus (LMV) strain Ls 1**					
(+)							
QL		absent				Bijou, Hilde II, ~~Salvina~~	1
		present				Corsica, Diveria	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
34. **(old 41)** **(+)**	**VG**	**Resistance to *Nasonovia ribisnigri* biotype Nr: 0**					
QL		absent				Abel, Green Towers, Nadine	1
		present				Barcelona, Dynamite, Silvinas	9
35. **(old 42)** **(+)**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *lactucae* race 1**					
QL		absent				Cobham Green, Patriot, ~~Salinas~~	1
		present				Costa Rica No. 4, Romasol	9

8. Explanations on the Table of Characteristics

8.1 Lettuce growth (sub-)types (under section 5.3)

Butterhead lettuce

Crisp lettuce
Iceberg

Crisp lettuce
Batavia

Crisp lettuce
Open heading Batavia

Cos lettuce

Grasse lettuce

Cutting lettuce
Frisée d'Amérique

Cutting lettuce
Oakleaf

Cutting lettuce
Oakleaf

Cutting lettuce
Frillice

Cutting lettuce
Lollo

Cutting lettuce
Divided

Cutting lettuce
Divided

Cutting lettuce
Multileaf

Novita lettuce

Stem lettuce

8.2 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 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.

(b) 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.

8.3 Explanations for individual characteristics

Ad. 3: Plant: head formation

1
no head

2
open head

3
closed head

(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.

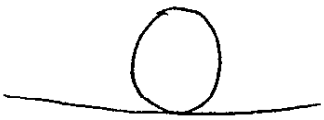
Ad. 4: Only cutting lettuce varieties: Plant: number of leaves

Observe number of leaves of the whole plant by cutting the stem out of the harvested plant.

Ad. 5: Leaf: attitude

1
erect

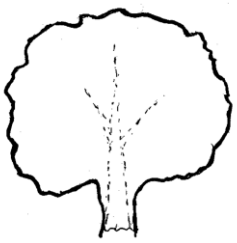
3
semi-erect



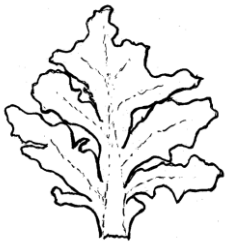
5
horizontal

Ad. 6: Leaf: division

Divided leaves have incisions more than halfway to the midrib.



1
entire



2
divided

Ad. 7: Only varieties with divided leaves: Leaf: number of divisions

To observe only the incisions more than halfway to the midrib. Incisions less than halfway to the midrib are to be described as incisions of the margin (Char. 22 and 23).

1
very small

3
small

5
medium

7
large

9
very large

Ad. 8: Only oakleaf sub-types: Leaf: width of lobes

3
narrow

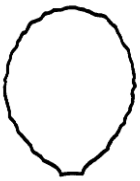
5
medium

7
broad

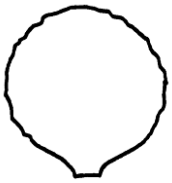
Ad. 9: Only varieties with entire leaves: Leaf: shape

1
narrow elliptic

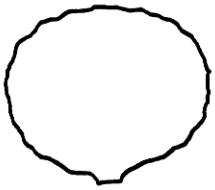
2
medium elliptic



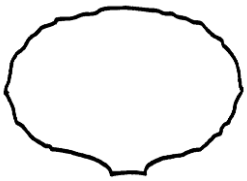
3
broad elliptic



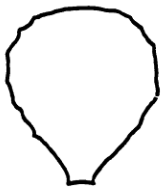
4
circular



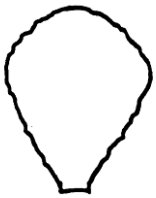
5
transverse broad elliptic



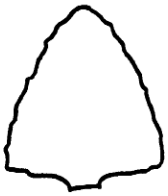
6
transverse narrow elliptic



7
obovate



8
broad obtrullate



9
triangular

Ad. 10: Only varieties with entire leaves: Leaf: cross section

1
Convex

3
flat

5
concave

Ad. 11: Leaf: anthocyanin coloration

In some cases, for example when the area of anthocyanin is very small or when environmental conditions are not optimal for anthocyanin coloration, a seedling test may be useful. Stressing seedlings under cold and dry conditions gives a clear and easy indication on presence or absence of anthocyanin.

Ad. 12: Leaf: area covered with anthocyanin coloration

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

Ad. 13: Leaf: hue of anthocyanin coloration
Ad. 14: Leaf: intensity of anthocyanin coloration

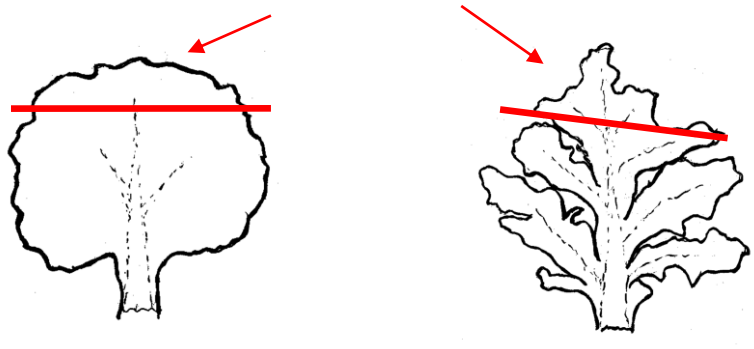
Intensity of anthocyanin coloration (Ch. 14)	Hue of anthocyanin coloration (Ch. 13)		
	1 reddish	2 brownish	3 purplish
1 very weak			
3 weak	Du bon jardinier, Steirer Krauthauptel	Brauner Trotzkopf, Diablo, Maravilla de Verano	
5 medium	Lollo rossa	Frisée d'Amérique, Luana, New Red Fire, Salad bowl rossa	
7 strong	Jadigon	Duplex, Merveille des quatre saisons	
9 very strong	Revolution	Multired 54	Faradia, Iride

Ad. 15: Leaf: hue of green color
Ad. 16: Leaf: intensity of green color

Only to describe for green varieties and for two-colored varieties with an area covered with anthocyanin smaller than large, so the green color of the leaf can be observed without picking a leaf from the plant.

Intensity of color (Ch. 16)	Hue of green color (Ch. 15)		
	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, Verdetrix		

Ad. 22: Leaf: depth of incisions on margin of apical part
Ad. 23: Leaf: density of incisions on margin of apical part



Ad. 24: Leaf: venation

1
not flabellate

2
semi flabellate

3
flabellate

Ad. 25: Only cutting lettuce varieties: Heart: density

Density of the leaves at the center of the plant. To be observed at full grown stage, just before deterioration or bolting.

Ad. 26: Only varieties with closed head: Head: degree of overlapping of upper part of leaves

1
very weak

3
weak

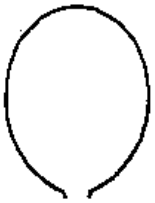
5
medium

7
strong

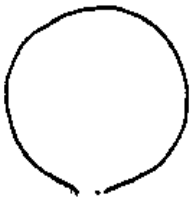
9
very strong

Ad. 28: Only varieties with closed head: Head: shape in longitudinal section

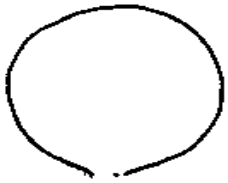
1
narrow elliptic



2
broad elliptic



3
circular



4
transverse broad elliptic

Ad. 30: Time of beginning of bolting under long day conditions

To be observed 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. 31: Plant: fasciation

To be observed at the bolted plant, 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. 32: Resistance to downy mildew (*Bremia lactucae*) (Bl:16-31)

*** 1. Pathogen**	***Bremia lactucae***
2. Quarantine status	-
* 3. Host species	*Lactuca sativa* L.
* 4. Source of inoculum	GEVES (France) or Naktuinbouw (The Netherlands)
*** 5. Isolate**	**Bl: ~~2,5,7,12,14,15,~~16, 17, 20-31** (see table below)
6. Establishment isolate identity	Test on differentials
7. Establishment pathogenicity	Test on susceptible varieties
8. Multiplication inoculum	
8.1 Multiplication medium	Lettuce leaf
8.2 Multiplication variety	Susceptible variety, for example Green Towers. For higher races, a variety with defeated resistance may be preferable to keep the isolate fit.
8.3 Plant stage at inoculation	Cotyledon to first leaf
8.4 Inoculation medium	Tap water
8.5 Inoculation method	Spraying a spore suspension
8.6 Harvest of inoculum	Washing off from leaves
8.7 Check of harvested inoculum	Counting spores
8.8 Shelf life/viability inoculum	2 hours at room temperature; 2 days in fridge
9. Format of the test	
* 9.1 Number of plants per genotype	Normally 60, minimum 20
* 9.2 Number of replicates	-
* 9.3 Control varieties	(Informative) differentials
* 9.4 Test design	Include control varieties
9.5 Test facility	Climate room
9.6 Temperature	15°C-17°C
9.7 Light	Adequate for good plant growth; seedlings should not etiolate. Reduced light 24 hours after inoculation
9.8 Season	-
9.9 Special measures	Plants may grow on wet blotting paper with or without a nutrient solution, or on potting soil. High humidity (>90%) is essential for infection and sporulation.
10. Inoculation	
10.1 Preparation inoculum	Washing off from leaves by vigorous shaking in a closed container
10.2 Quantification inoculum	Counting spores ; spore density should be 3.10^4-1.10^5
*10.3 Plant stage at inoculation	Cotyledon stage
*10.4 Inoculation method	Spraying till run-off Reduced light 24 hours after inoculation
10.5 First observation	7 days after inoculation
10.6 Second observation	10 days after inoculation
*10.7 Final observations	13 days after inoculation; two of these three time points may be sufficient. The day of maximum sporulation should occur in this period.
11. Observations	
*11.1 Method	Visual observation of sporulation and necrotic reaction to infection
*11.2 Observation scale	+ 1. Abundant sporulation on both sides of the cotyledon (+) 2. Normal sporulation on the lower side of the cotyledon 3. Normal sporulation on the lower side of the

cotyledons combined with necrotic spots
(-) 4. Sparse sporulation on the lower side of the cotyledons combined with necrosis
(-) 5. Necrotic pinpoints
- 6. No symptoms

*11.3 Validation of test

On standards. In case of a large percentage of plants in class 3 a retest is needed.

11.4 Off-types

Plants in class 1 or 2 in a resistant variety
Plants in class 4, 5 or 6 in a susceptible variety
3 or less off-types in 60 plants

*12. Interpretation of data

Class 1, 2 ~~and 3~~: susceptible
Class 3: undecided
Class 4, 5 and 6: resistant

13. Critical control points:

Reaction of standards. The infection pressure may vary between experiments, leading to slight differences in sporulation intensity. When the reactions are not clear the experiment should be repeated.

For reference: The international Bremia evaluation board (IBEB) produces regular updates of the host differential reaction table. The most recent table is available through ISF at www.worldseed.org. The table for isolates mentioned in this guideline is given below.

	Green Towers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
		Lednicky	UC DM2	Diandie	R4T57D	Valmaine	Sabine	LSE 57/15	UC DM10	Capitan	Hilde II	Pennlake	UC DM14	NunDm15	CGDm 16	NunDm17	Cdorado	Ninja	Discovery	Angelès	RYZ 2164	RYZ 910457	Bedford	Balesta	Bellissimo
Bl: 16	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
Bl: 17	+	-	+	+	-	+	-	+	+	-	+	+	+	+	-	-	+	-	+	-	-	-	(+)	(-)	-
Bl: 20	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-	-	(-)
Bl: 21	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	(-)	(-)	-	-
Bl: 22	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	(-)	-	-	(-)	+	-
Bl: 23	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-
Bl: 24	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-	-	-	-	-
Bl: 25	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	(-)	-	-	-	-	-
Bl: 26	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	-
Bl: 27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	(-)	+	-
Bl: 28	+	+	+	-	+	+	+	+	+	+	+	+	(-)	-	+	-	+	-	-	+	-	+	-	-	(-)
Bl: 29	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-
Bl: 30	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	-	-
Bl: 31	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	+	-	-	-

Ad. 33: Resistance to lettuce mosaic virus (LMV)

1. Pathogen	**Lettuce mosaic virus**
2. Quarantine status	No
* 3. Host species	Lettuce - *Lactuca sativa*
* 4. Source of inoculums	Isolate collection at Naktuinbouw
* 5. Strain	Ls1
6. Establishment isolate identity	resistant and susceptible controls
7. Establishment pathogenicity	susceptible control inoculation
8. Multiplication inoculums	
8.1 Multiplication medium	
8.2 Multiplication variety	susceptible control
8.3 Plant stage at inoculation	2-3 leaves
8.4 Inoculation medium	0,05 M PBS, 0,25% (w/v) Na_2SO_3 0,5% $C_5H_{10}NNaS_2.3H_2O$, 4% carborundum and 5% active charcoal
8.5 Inoculation method	rubbing; repeat this 4 after 4 d; 1-2 h high humidity after inoculation
8.6 Harvest of inoculums	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 inoculums	compare with mock inoculation with LMV buffer + carborundum+ charcoal
8.8 Shelf life/viability inoculums	2 h at 4°C or on ice
9. Format of the test	
* 9.1 # plants per genotype	at least 20
* 9.2 # replicates	1
* 9.3 Control varieties	R = Corsica, Diveria; S = Bijou, Hilde II, Sprinter
* 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, then 23/18°C d/n, after second inoculation again 15°C
9.7 Light	16/8 h d/n; light ca. 5000 lux
9.8 Season	
9.9 Special measures	
10. Inoculation	
10.1 Preparation inoculum	fresh leaf ground in fresh LMV buffer incl. carborundum and active charcoal
10.2 Quantification inoculum	No
*10.3 Plant stage at inoculation	1st inoculation, 4d later 2nd inoculation
*10.4 Inoculation method	rubbing, rinse carborundum off
*10.5 End of test	21 dpi for red lettuce; 14 dpi for green lettuce
11. Observations	
*11.1 Method	Visual estimate of mosaic severity. Compare with standards.
*11.2 Observation scale	Resistant standard = no symptoms Susceptible standard = growth retardation, young leaves with mosaic, leaf curling
*11.3 Validation of test	Standards should conform to description
11.4 Off-types	
*12. Interpretation of data	Classify R or S per plant Comparisons preferably within between standards of same crop type

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. Red anthocyan color in leaves may mask mosaic symptoms

Ad. 34: Resistance to *Nasonovia ribisnigri* (Nr:0)

*1. **Pathogen**	***Nasonovia ribisnigri***
2. Quarantine status	no
* 3. Host species	*Lactuca sativa* (Lettuce)
* 4. Source of inoculum	Naktuinbouw
* 5. Isolate	Nr:0 (non-resistance breaking), red coloured biotype
6. Establishment isolate identity	the ends of the legs are black, size 1.5-2.5 mm
7. Establishment pathogenicity	with susceptible control Abel
8. **Multiplication inoculum**	
8.1 Multiplication medium	-
8.2 Multiplication variety	Abel
8.3 Plant stage at inoculation	4 leaves
8.4 Inoculation medium	-
8.5 Inoculation method	transfer ~5 aphids per plant with a fine painting brush
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 # plants per genotype	28
* 9.2 # replicates	no replicates
* 9.3 Control varieties	**Susceptible: Abel, Nadine** **Resistant: Dynamite, Barcelona**
* 9.4 Test design	no
9.5 Test facility	Glasshouse
9.6 Temperature	12°C for germination and early growth
9.7 Light	daylight
9.8 Season	temperature 20-22°C, keep below 26°C
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
10.2 Quantification inoculum	-
*10.3 Plant stage at inoculation	15 d old lettuce seedlings
*10.4 Inoculation method	transfer 5 small or medium sized aphids to each plant
10.5 First observation	10 d post inoculation
10.6 Second observation	daily check after first observation
*10.7 End of test	max. 15 d 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% (2/28 plants) plants are undecided or off-type, the experiment should be repeated
11.4 Off-types	-
*12. Interpretation of data	**0 or 1 Resistant** **2 Undecided** **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 dpi. Only adult, red aphids are counted; young aphids are transparent and do not count

Ad. 35: 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	*Lactuca sativa* L.
4. Source of inoculum	NIAS Genebank (JP), INRAN (IT), Naktuinbouw (NL), GEVES (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)
8.3 Plant stage at inoculation	see 10.3
8.5 Inoculation method	see 10.4
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	20 plants
9.2 Number of replicates	
9.3 Control varieties	
Susceptible	Cobham Green, ~~Salinas,~~ Patriot Cobham Green is slightly less ~~and Salinas is less~~ susceptible than Patriot
Resistant to Fol: 1	Costa Rica No.4, Romasol
9.4 Test design	include control varieties
9.5 Test facility	greenhouse or climate room
9.6 Temperature	20-28 ^{o}C
9.7 Light	under natural day length
10. Inoculation	
10.1 Preparation inoculum	inoculation by sowing on contaminated soil: Wheat bran-soil medium culture are mixed with sterilized soil inoculation by soaking seedlings: soaking of roots and of hypocotyls axis for 5 to 15 min in the inoculums 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^{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
10.4 Inoculation method	two methods can be used for inoculation: by sowing seeds to contaminated soil or by soaking seedlings
10.5 First observation	after 7- 10 days from inoculation
10.6 Second observation	14 days from inoculation
10.7 Final observations	20-25 days after inoculation (sowing or soaking)
11. Observations	
11.1 Method	visual and/or counting number of plants with symptom
11.2 Observation scale	inoculation by sowing on contaminated soil: symptoms: stunting, wilting, dead plant as reference calculate of Disease Severity Index (DSI) and Disease Incidence(DI) 0: healthy 1: slightly stunting, growing reduction 2: severely stunting

3: die
DSI = (0A + 1B + 2C + 3D) / (A + B + C + D)
*A ~ D: number of plants of each category
DI = (0A + 1B + 2C + 3D) *100/ ((A + B + C + D)*3)
inoculation by soaking seedlings:
symptoms: growth reduction and brown vessels above cotyledons, dead plant

11.3 Validation of test..... analysis of results should be calibrated with results of controls.

12. Interpretation of data..... inoculation by sowing on contaminated soil:
susceptible: severely stunting, wilting, dead plant
(DSI :Relative evaluation to DSI of example variety)
(Race1:DI Value is higher than 10%)
resistant: no stunting, no wilting
(DSI: relative evaluation to DSI of example variety), (Race1: DI value is lower than 10%)
inoculation by soaking seedlings:
susceptible: growth reduction and brown vessels above cotyledons, dead plant
resistant: no growth reduction and no brown vessels above cotyledons

13. Critical control points

Availability of *Fusarium oxysporum* f.sp. *lactucae* race 1

NIAS: National Institute of Agrobiological Sciences
2-1-2, Kannondai, Tsukuba, Ibaraki,305-8602, Japan
Tel: +81-29(838)7406, fax: +81-29(838)7408,
E-mail: genebank@nias.affrc.go.jp
http://www.gene.affrc.go.jp/about_en.php

INRAN: National Research Institute for Food and Nutrition
Loc. Corno d'Oro SS 18, km 77.70 – 84091 Battipaglia (SA) Italy
Tel: +39 0828 309484, fax +39 0828 302382, E-mail: romana.bravi@entecra.it
http://www.ense.it

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

GEVES: Groupe d'Etude et de Contrôle des Variétés et des Semences
25 Rue Georges Morel, CS 90 024, 49071 Beaucouzé Cedex, France
Valerie.GRIMAULT@geves.fr

9. Literature

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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 [ ]
(please state parent varieties)

(……..……..…) x (……..……..…)
female parent male parent

(b) partially known cross [ ]
(please state known parent variety(ies))

(……..……..…) x (……..……..…)
female parent male parent

(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)

4.2 Method of propagating the variety

(a) Self-pollination [ ]

(b) Other [ ]
(please provide details)

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

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	**Growth (sub-)types (according to Section 8.1 of the Test Guidelines)**			
	Butterhead lettuce		Clarion, Maikönig	[ ]
	Crisphead lettuce	Iceberg	Great Lakes 659, Roxette, Saladin, Vanguard 75	[ ]
		heading Batavia	Curtis, Masaida, Visyon	[ ]
		open heading Batavia	Aquarel, Funnice	[ ]
	Cos lettuce		Actarus, Blonde maraîchère, Pinokkio,	[ ]
	Grasse lettuce		Craquerelle du Midi, Sucrine, Xanadu	[ ]
	Cutting lettuce	Frisée d'Amérique	Bijou, Faradia, Grand Rapids	[ ]
		Oakleaf	Catalogna, Kipling, Muraï, Salad Bowl	[ ]
		Frillice	Frilett	[ ]
		Lollo	Lollo rossa, Revolution	[ ]
		Divided	Curletta, Duplex, Jadigon, Rodagio	[ ]
		Multileaf	Felluca , Sartre, Xeres	[ ]
	Novita lettuce		Norvick	[ ]
	Stem lettuce		Celtuce	[ ]
5.2 (1)	**Seed: color**			
	white		Verpia	1[ ]
	yellow		Durango	2[ ]
	black		Kagraner Sommer 2	3[ ]
5.3 (11)	**Leaf: anthocyanin coloration**			
	absent		Clarion	1[ ]
	present		Lollo rossa	9[ ]

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

	Characteristics	Example Varieties	Note
5.4 (14)	**Leaf: intensity of anthocyanin coloration**		
	very weak	~~Chicon de Charentes, Muranta, Rumina~~	1[ ]
	very weak to weak		2[ ]
	weak	Du bon jardinier	3[ ]
	weak to medium		4[ ]
	medium	Lollo rossa, Luana, ~~Trocadéro à graine noire~~	5[ ]
	medium to strong		6[ ]
	strong	~~Amandine,~~ Merveille des quatre saisons	7[ ]
	strong to very strong		8[ ]
	very strong	~~Little Leprechaun,~~ Iride, Revolution	9[ ]
5.5 (15)	**Leaf: hue of green color (only for (partly) greenish varieties)**		
	absent	~~Donatello,~~ Verpia	1[ ]
	yellowish	Dorée de printemps	2[ ]
	greyish	Celtuce, Du bon jardinier	3[ ]
5.6 (16)	**Leaf: intensity of green color (only for (partly) greenish varieties)**		
	very light		1[ ]
	very light to light		2[ ]
	light	Blonde maraîchère, Lollo	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[ ]

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

	Characteristics	Example Varieties	Note
5.4 (30)	**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	~~Carélia,~~ Pantlika	5[ ]
	medium to late		6[ ]
	late	Hilde II	7[ ]
	late to very late		8[ ]
	very late	Erika, ~~Kinemontepas, Rex,~~ Roxette	9[ ]
5.6 (32.1)	**Resistance to downy mildew (*Bremia lactucae*) Isolate Bl: 16**		
	absent	Green Towers	1[ ]
	present	Argelès, Ninja	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	*Plant: diameter*	*medium*	*medium to large*

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

7.3.1 Special conditions for the examination of the variety

Type of culture:

- in glasshouse [ ]

- in the open [ ]

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.

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

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

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

[End of document]