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# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

[1]

**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*
*Enlarged Editorial Committee*
*at its meeting, to be held in Geneva,*
*from 2017-01-11 to 2017-01-12*

*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 *Bremia lactucae* (Bl) isolate Bl: 16 (characteristic 38)

In the first place, the collection should be divided according to types as mentioned in the Table 1on the next page below. In cases of doubt to which type a variety belongs to, it should be tested in all relevant types.

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

Table 1

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

## 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<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>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<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **1.** | **(*)** | **PQ VG** | | | | | |
| | | **Seed: color** | | | | | |
| | | white | | | | Verpia | 1 |
| | | yellow | | | | Durango | 2 |
| | | brown | | | | Oaklin | 3 |
| | | black | | | | Kagraner Sommer 2 | 4 |
| **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)** | | | | |
| | | **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 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **5.** | | **QN VG** | **(+) (c)** | | | | |
| | | **Leaf: attitude** | **Feuille : port** | **Blatt: Haltung** | **Hoja: porte** | | |
| | | erect | dressé | aufrecht | erecto | Feria, Pinokkio | 1 |
| | | semi-erect | demi-dressé | halbaufrecht | semierecto | Expedition, Sartre | 3 |
| | | horizontal | horizontal | waagerecht | horizontal | Divina | 5 |
| **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.** | | **PQ VG** | **(+) (c)** | | | | |
| | | **<u>Only varieties with divisions absent or very few</u>: 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 |
| | | broad obtrullate | | | | | 10 |
| | | obovate | | | | Raisa | 11 |
| | | oblanceolate | | | | Xiangshengcai | 12 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **8.** | | **PQ VG** | **(+) (c)** | | | | |
| | | **<u>Only varieties with divisions absent or very few</u>: Leaf: shape of apex** | | | | | |
| | | acute | | | | Celtuce | 1 |
| | | obtuse | | | | Actarus | 2 |
| | | rounded | | | | Blonde maraîchère, Maserati | 3 |
| | | obcordate | | | | PS 6545691 | 4 |
| **9.** | | **QN VG** | **(c)** | | | | |
| | | **<u>Only varieties with divisions absent or very few</u>: Leaf: cross section** | **Feuille : forme en section transversale** | **Blatt: Form im Querschnitt** | **Hoja: forma en sección transversal** | | |
| | | concave | concave | konkav | cóncava | Sunstar | 1 |
| | | flat | plate | flach | plana | Clarion, Lollo rossa | 3 |
| | | convex | | | | Tiago | 5 |
| **10.** | | **QN VG** | **(+) (c)** | | | | |
| | | **<u>Only Oakleaf type varieties</u>: Leaf: width of lobes** | | | | | |
| | | narrow | | | | Kibrille, Rougini | 3 |
| | | medium | | | | Bandolin, Ribaï | 5 |
| | | broad | | | | Horix, Starix, Vizir | 7 |
| **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 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **12.** | **(*)** | **PQ VG** | **(+) (c)** | | | | |
| | | **Leaf: hue of anthocyanin coloration** | | | | | |
| | | reddish | | | | Lollo rossa | 1 |
| | | purplish | | | | Iride | 2 |
| | | brownish | | | | Luana, Maravilla de Verano | 3 |
| **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: 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 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **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** | **Feuille: épaisseur** | **Blatt: Dicke** | **Hoja: espesor** | | |
| | | thin | mince | dünn | delgado | Bijou, Lollo rossa, Raisa | 3 |
| | | medium | moyen | mittel | medio | Curtis, Expedition | 5 |
| | | thick | épais | dick | grueso | Frilett, Roxette | 7 |
| **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 | 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** | **Feuille : ondulation du bord** | **Blatt: Randwellung** | **Hoja: ondulación del borde** | | |
| | | absent or very weak | absente ou très faible | fehlend oder sehr gering | ausente o muy débil | Tiago | 1 |
| | | weak | faible | gering | débil | Commodore | 3 |
| | | medium | moyenne | mittel | media | Noisette, Pentared | 5 |
| | | strong | forte | stark | fuerte | Calmar, Invicta | 7 |
| | | very strong | très forte | sehr stark | muy fuerte | Lollo rossa | 9 |

| | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|
| **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 |
| **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 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **25.** | **(*)** | **QN** **VG** | **(+)** **(a), (c)** | | | | |
| | | **Leaf: venation** | | | | | |
| | | not flabellate | | | | Verpia, Xanadu | 1 |
| | | semi flabellate | | | | Kibrille, Muraï | 2 |
| | | flabellate | | | | Locarno, Roxette | 3 |
| **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.** | **(*)** | **QN** **MS/VG** | **(+)** **(a), (b)** | | | | |
| | | **Only varieties with degree of overlapping of upper part of leaves medium or strong: Head: shape in longitudinal section** | | | | | |
| | | narrow elliptic | | | | Verte maraîchère | 1 |
| | | broad elliptic | | | | Amadeus, Sucrine | 2 |
| | | circular | | | | Verpia | 3 |
| | | narrow oblate | | | | 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 |

| | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|
| **29.** | **QN MS/VG** | **(+) (b)** | | | | |
| | **Only Stem type varieties: Stem: length** | | | | | |
| | short | | | | Wuweijianye | 3 |
| | medium | | | | Zipixiang | 5 |
| | long | | | | Guasihong | 7 |
| **30.** | **QN MS/VG** | **(+) (b)** | | | | |
| | **Only Stem type varieties: Stem: width** | | | | | |
| | 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<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **34.** | | **QN MG/VG** | | | | | |
| | | **<u>Only varieties with degree of overlapping of upper part of leaves medium or strong</u>: 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<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|
| **38.** | **QL** **VG** | **(+)** | | | | |
| | **Resistance to *Bremia lactucae* (Bl) isolate Bl: 16** | | | | | |
| | absent | | | | Green Towers | 1 |
| | present | | | | Argelès | 9 |
| **39.** | **QL** **VG** | **(+)** | | | | |
| | **Resistance to *Bremia lactucae* (Bl) isolate Bl: 17** | | | | | |
| | absent | | | | Green Towers | 1 |
| | present | | | | Argelès | 9 |
| **40.** | **QL** **VG** | **(+)** | | | | |
| | **Resistance to *Bremia lactucae* (Bl) isolate Bl: 20** | | | | | |
| | absent | | | | Green Towers | 1 |
| | present | | | | FrRsal-1 | 9 |
| **41.** | **QL** **VG** | **(+)** | | | | |
| | **Resistance to *Bremia lactucae* (Bl) isolate Bl: 21** | | | | | |
| | absent | | | | Green Towers | 1 |
| | present | | | | Argelès, Colorado | 9 |
| **42.** | **QL** **VG** | **(+)** | | | | |
| | **Resistance to *Bremia lactucae* (Bl) isolate Bl: 22** | | | | | |
| | absent | | | | Green Towers | 1 |
| | present | | | | FrRsal-1 | 9 |
| **43.** | **QL** **VG** | **(+)** | | | | |
| | **Resistance to *Bremia lactucae* (Bl) isolate Bl: 23** | | | | | |
| | absent | | | | Green Towers | 1 |
| | present | | | | Colorado | 9 |

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

| | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|
| **50.** | **QL VG** | **(+)** | | | | |
| | **Resistance to *Bremia lactucae* (Bl) 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 MS/VG** | **(+)** | | | | |
| | **Resistance to *Nasonovia ribisnigri* (Nr) 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* (Fol) 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.

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

**Butterhead type**


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

**Novita type**

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

**Iceberg type**


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

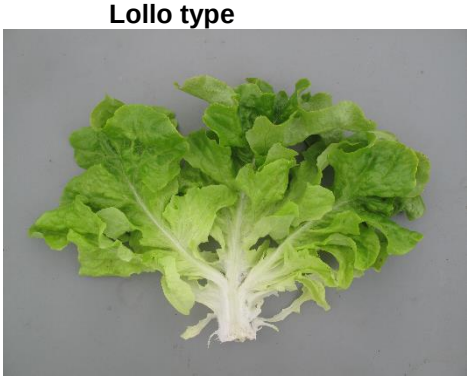
**Batavia type**

**Frisée d'Amérique type**

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

**Lollo type**

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



**Oakleaf type**

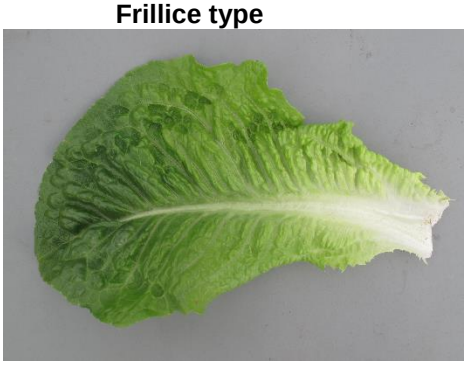
Thin, divided leaves; divisions have an oakleaf or lobed shape with in general a rounded tip. Radichetta or Catalogna with acute tip of the division. Heart can be loose to dense.


**Multi-divided type**

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

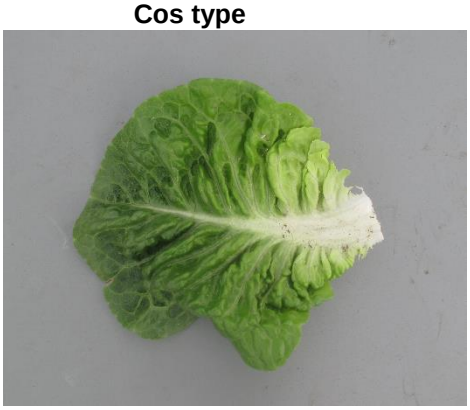

**Frillice type**

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

**Cos type**

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



**Gem 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.


**Stem 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.

(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

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

3
few

5
medium

7
many

Ad. 5: Leaf: attitude

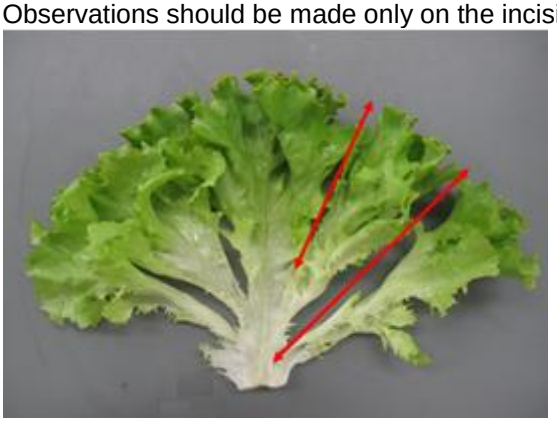
1
erect

3
semi-erect

5
horizontal

Ad. 6: Leaf: number of divisions

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



1
absent or very few

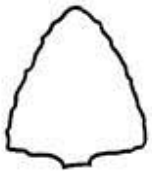
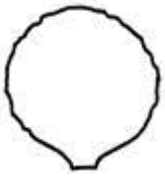
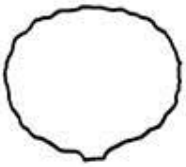
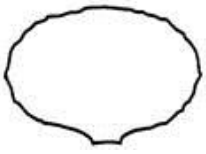
3
few

5
medium

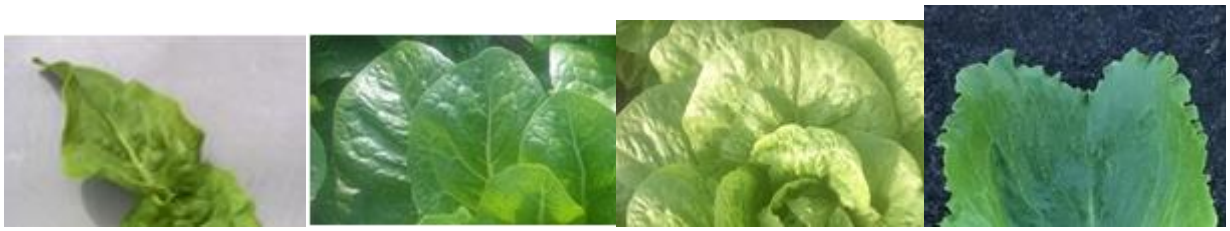
7
many

9
very many

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

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

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



1 acute | 2 obtuse | 3 rounded | 4 obcordate

Ad. 9: Only varieties with divisions absent or very few: Leaf: cross section

1 concave | 3 flat | 5 convex

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

3 narrow | 5 medium | 7 broad

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

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

Ad. 13: Leaf: area covered by anthocyanin coloration

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

3
small

5
medium

7
large

9
very large

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

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

| Intensity of green color (Char. 15) | Color (Char. 14) | | |
|---|---|---|---|
| | 1<br>green | 2<br>yellowish green | 3<br>greyish green |
| 1<br>very light | | | |
| 3<br>light | Blonde maraîchère, New Red Fire | Lollo Bionda, Steirer Krauthauptel | Celtuce |
| 5<br>medium | Ballerina | Aquarel, Australische Gele, Dorée de printemps | Clarion, Du bon jardinier, Durango |
| 7<br>dark | Actarus, Baby Star, Expedition, Verpia | | Webbs Wonderful |
| 9<br>very dark | Pascal, Verdetrix | | |

Ad. 19: Leaf: size of blisters

Observations should be made on the whole leaf.

3
small

5
medium

7
large

Ad. 20: Leaf: undulation of margin

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

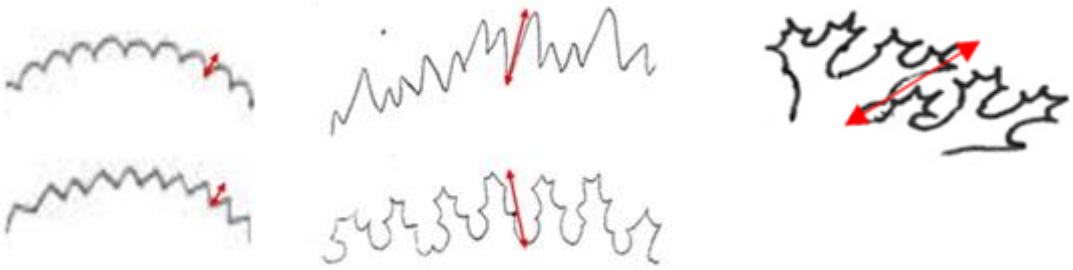
Ad. 21: Leaf: type of incisions of margin

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

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

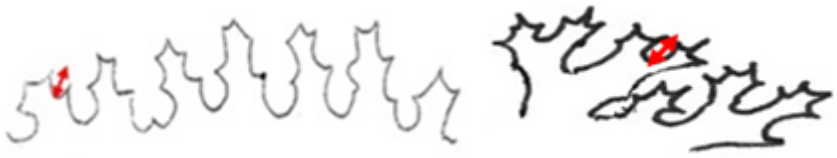
Ad. 22: Leaf: depth of incisions of margin

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 observations should not be made on 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.

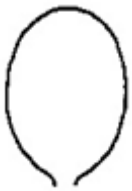
Ad. 25: Leaf: venation

1
not flabellate

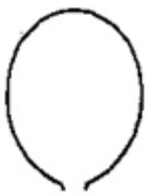
2
semi flabellate

3
flabellate

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

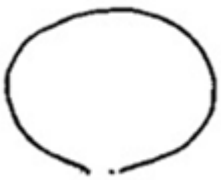


1
narrow elliptic



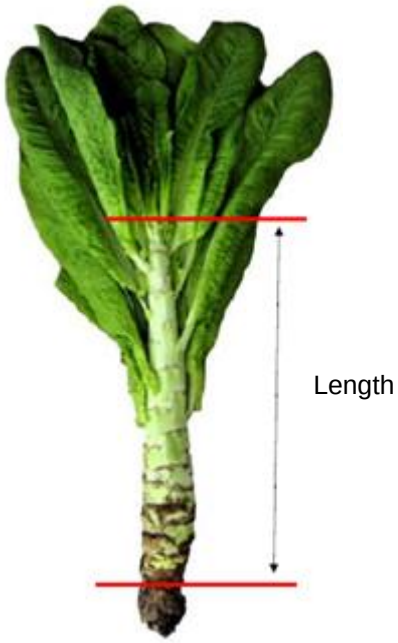
2
broad elliptic

3
circular



4
narrow oblate

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




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

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 to 50: Resistance to *Bremia lactucae* (Bl), several isolates

| | |
|---|---|
| 1. Pathogen | *Bremia lactucae* |
| 2. Quarantine status | no |
| 3. Host species | lettuce - *Lactuca sativa* L. |
| 4. Source of inoculum | GEVES[2] (FR) or Naktuinbouw[3] (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.<br>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.<br>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).<br>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.<br>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<br>two of these three observations may be sufficient, the third notation is optional for observation of evolution of symptoms in case of doubt.<br>the day of maximum sporulation should occur in this period. |
| 11. Observations | |
| 11.1 Method | visual observation of sporulation and necrotic reaction to infection |

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| | |
|---|---|
| 11.2 Observation scale | resistant:<br>0 no sporulation, no necrosis<br>1 no sporulation, necrosis present<br>2 weak sporulation (much less than susceptible control) with necrosis<br>3 weak sporulation (less than susceptible control and not evolving between second and third observation) with necrosis<br>4 very sparse sporulation (not evolving between second and third observation) without necrosis<br><br>susceptible:<br>5 reduced sporulation (compared to susceptible control) without necrosis<br>6 normal sporulation without necrosis |
| 11.3 Validation of test | on standards<br>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<br>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.<br>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.<br>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 | 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 | + | + | + | + | - | - | + | - | - | + | + | - | - | - | + |

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[4] (FR) or Naktuinbouw[5] (NL) |
| 5. Isolate | pathotype II (isolates LMV-0 and Ls1 belong to the same pathotype) |
| 6. Establishment isolate identity | resistant and susceptible controls |
| 7. Establishment pathogenicity | susceptible control inoculation |
| 8. Multiplication inoculum | |
| 8.2 Multiplication variety | susceptible control |
| 8.3 Plant stage at inoculation | 2-3 leaves |
| 8.4 Inoculation medium | 0,05 M PBS, 0,25% (w/v) $Na_2SO_3$ 0,5% $C_5H_{10}NNaS_2.3H_2O$, 4% carborundum and 5% active charcoal |
| 8.5 Inoculation method | rubbing; optionally repeat after 4 d; 1-2 h high humidity after inoculation |
| 8.6 Harvest of inoculum | homogenized fresh leaf in buffer (50% w/v);<br>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)<br>resistant: Capitan (green), Corsica (green), Diveria (red) |
| 9.4 Test design | several mock-inoculated plants in the same tray |
| 9.5 Test facility | climate chamber |
| 9.6 Temperature | after inoculation 15-22°C |
| 9.7 Light | 12-16 h light ca. 5000 lux |
| 10. Inoculation | |
| 10.1 Preparation inoculum | fresh leaf ground in fresh LMV buffer incl. carborundum and active charcoal |
| 10.3 Plant stage at inoculation | 1st leaf well-developed at 1st inoculation, optionally 4 days later 2nd inoculation |
| 10.4 Inoculation method | rubbing, rinse carborundum off |
| 10.7 Final observations | 21 days post inoculation |
| 11. Observations | |
| 11.1 Method | visual estimate of mosaic severity; compare with standards, preferably with standards of same growth type. |
| 11.2 Observation scale | resistant = no symptoms<br>susceptible = growth retardation, young leaves with mosaic, leaf curling |
| 11.3 Validation of test | standards should conform to description |
| 12. Interpretation of data in terms of UPOV characteristic states | classify resistant or susceptible per plant, see 11.2. |
| 13. Critical control points | Sprinter is less susceptible than many other susceptible varieties, this variety can be used to detect low inoculation pressure in a specific experiment.<br>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* (Nr) biotype Nr: 0

| | |
|---|---|
| 1. Pathogen | *Nasonovia ribisnigri* |
| 2. Quarantine status | no |
| 3. Host species | lettuce - *Lactuca sativa* L. |
| 4. Source of inoculum | Naktuinbouw[6] (NL) |
| 5. Isolate | Nr: 0, preferably red 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<br>resistant: Barcelona, Bedford, Dynamite, Silvinas |
| 9.4 Test design | |
| 9.5 Test facility | glasshouse |
| 9.6 Temperature | after inoculation: 20-22°C, keep below 26°C |
| 9.7 Light | daylight |
| 9.9 Special measures | containment of winged aphids needs special attention |
| 10. Inoculation | |
| 10.1 Preparation inoculum | transfer by shake-off or with brush into Petri-dish |
| 10.3 Plant stage at inoculation | 2 to 3 week old seedlings |
| 10.4 Inoculation method | transfer 5 small or medium sized aphids to each plant |
| 10.7 Final observations | 15 to 20 days post inoculation |
| 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<br>1 1-5 aphids<br>2 6-10 aphids<br>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<br>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.<br>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* (Fol) race 1

| | |
|---|---|
| 1. Pathogen | *Fusarium oxysporum* f.sp*. lactucae* |
| 2. Quarantine status | EPPO alert list |
| 3. Host species | lettuce - *Lactuca sativa* L. |
| 4. Source of inoculum | NIAS Genebank[7] (JP), CREA-SCS[8] (IT), Naktuinbouw[9] (NL), GEVES[10] (FR) |
| 5. Isolate | Fol: 1 |
| 6. Establishment isolate identity | use microscope and inoculation to lettuce susceptible standard |
| 7. Establishment pathogenicity | use lettuce susceptible standard |
| 8. Multiplication inoculum | |
| 8.1 Multiplication medium | inoculation by sowing on contaminated soil: Wheat bran-soil medium<br>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<br>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)<br>moderately resistant: Affic, Fuzila, Natexis (Natexis is the lower level of moderate resistance)<br>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 $^{o}$C (day) / 20 $^{o}$C (night) |
| 9.7 Light | under natural day length |
| 10. Inoculation | two methods can be used for inoculation: |

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

| | |
|---|---|
| 10.4 Inoculation method | two methods can be used, as described above |
| 10.5 First observation | 7- 10 days post inoculation |
| 10.6 Second observation | 14 days post inoculation |
| 10.7 Final observations | 20-25 days post inoculation (sowing or soaking). One or two of these 3 observations may be sufficient. The observation for inoculation by soaking is destructive since stems are cut for the observation of vessels. |
| 11. Observations | |
| 11.1 Method | visual and/or counting number of plants with symptom; as information calculate a disease index. |

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11.2 Observation scale

| **inoculation by sowing seeds on contaminated soil** | **inoculation by soaking seedlings** |
|---|---|
| 0: healthy | 0: plant without symptoms and healthy vessels  |
| 1: slightly stunting, growing reduction | 1: plant with brown vessels only below the cotyledon without yellowing and wilting  |
| 2: severely stunting | 2: plant with brown vessels above the cotyledon, without yellowing and wilting  |
| 3: die | 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.<br>a disease index may be helpful (example for the method of inoculation by soaking seedlings: DI= (0A + 1B + 2C + 3D + 4E) / (A + B + C + D + E), where A to E are number of plants in each category). |
| 12. Interpretation of data in terms of UPOV characteristic states | compare the distribution over the categories with the result of the controls. |

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10. Technical Questionnaire

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

| | |
|---|---|
| | Application date:<br>(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.

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

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 (1)** | **Seed: color** | | |
| | white | Verpia | 1 [ ] |
| | yellow | Durango | 2 [ ] |
| | brown | Oaklin | 3 [ ] |
| | black | Kagraner Sommer 2 | 4 [ ] |
| **5.2 (11)** | **Leaf: anthocyanin coloration** | | |
| | absent or very weak | Clarion | 1 [ ] |
| | very weak to weak | | 2 [ ] |
| | weak | Du bon jardinier | 3 [ ] |
| | weak to medium | | 4 [ ] |
| | medium | Lollo rossa, Luana | 5 [ ] |
| | medium to strong | | 6 [ ] |
| | strong | Merveille des quatre saisons | 7 [ ] |
| | strong to very strong | | 8 [ ] |
| | very strong | Iride, Revolution | 9 [ ] |
| **5.3 (15)** | **Leaf: intensity of green color** | | |
| | very light | | 1 [ ] |
| | very light to light | | 2 [ ] |
| | light | Blonde maraîchère, Lollo Bionda | 3 [ ] |
| | light to medium | | 4 [ ] |
| | medium | Aquarel, Clarion | 5 [ ] |
| | medium to dark | | 6 [ ] |
| | dark | Expedition, Verpia | 7 [ ] |
| | dark to very dark | | 8 [ ] |
| | very dark | Pascal, Verdetrix | 9 [ ] |

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

| | Characteristics | Example Varieties | Note |
|---|---|---|---|
| **5.4 (35)** | **Time of beginning of bolting** | | |
| | very early | Blonde à couper améliorée | 1 [ ] |
| | very early to early | | 2 [ ] |
| | early | Gotte à graine blanche | 3 [ ] |
| | early to medium | | 4 [ ] |
| | medium | Pantlika | 5 [ ] |
| | medium to late | | 6 [ ] |
| | late | Hilde II | 7 [ ] |
| | late to very late | | 8 [ ] |
| | very late | Erika, Roxette | 9 [ ] |

| 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

Type (see 5.3 and 8.1 in the Test Guidelines for Lettuce (document TG/13/11) 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 | [ ] |

Resistances:

(38) Resistance to *Bremia lactucae* (Bl) isolate Bl: 16
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(39) Resistance to *Bremia lactucae* (Bl) isolate Bl: 17
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(40) Resistance to *Bremia lactucae* (Bl) isolate Bl: 20
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(41) Resistance to *Bremia lactucae* (Bl) isolate Bl: 21
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(42) Resistance to *Bremia lactucae* (Bl) isolate Bl: 22
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(43) Resistance to *Bremia lactucae* (Bl) isolate Bl: 23
not tested 0 [ ] absent 1 [ ] present 9 [ ]

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

(44) Resistance to *Bremia lactucae* (Bl) isolate Bl: 24
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(45) Resistance to *Bremia lactucae* (Bl) isolate Bl: 25
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(46) Resistance to *Bremia lactucae* (Bl) isolate Bl: 26
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(47) Resistance to *Bremia lactucae* (Bl) isolate Bl: 27
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(48) Resistance to *Bremia lactucae* (Bl) isolate Bl: 29
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(49) Resistance to *Bremia lactucae* (Bl) isolate Bl: 30
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(50) Resistance to *Bremia lactucae* (Bl) isolate Bl: 31
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(51) Resistance to *Lettuce mosaic virus* (LMV) pathotype II
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(52) Resistance to *Nasonovia ribisnigri* (Nr) biotype Nr: 0
not tested 0 [ ] absent 1 [ ] present 9 [ ]

(53) Resistance to *Fusarium oxysporum* f. sp. *lactucae* (Fol) race 1
not tested 0 [ ] susceptible 1 [ ] moderately resistant 2 [ ] highly resistant 3 [ ]

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

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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

[End of document]