

Technical Working Party for Vegetables

TWV/57/12

Fifty-Seventh Session

Antalya, Türkiye, May 1 to 5, 2023

Original: English

Date: March 17, 2023

PARTIAL REVISION OF THE TEST GUIDELINES FOR LETTUCE

Document prepared by an expert from the Netherlands

Disclaimer: this document does not represent UPOV policies or guidance

- The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Lettuce (document TG/13/11 Rev. 2).
- The Technical Working Party for Vegetables (TWV), at its fifty-sixth session¹, agreed that the Test Guidelines for Lettuce (*Lactuca sativa* L.) be partially revised (see document TWV/56/22 “Report”, Annex II).
- The following changes are proposed:
 - Addition of “Resistance to *Bremia lactuca* (BI) Isolate BI: 36EU”, including example varieties;
 - Revision of explanation Ad. 38 to 52: Resistance to *Bremia lactuca* (BI).
 - Inclusion of characteristics from the Table of Characteristics in the Technical Questionnaire (see documents TWV/56/22 “Report”, paragraphs 102 and 103, and TWP/7/6 “Revision of Test Guidelines”)
- The proposed changes are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

Proposed addition of “Resistance to *Bremia lactuca* (BI) Isolate BI: 36EU”

Proposed addition

| | English | français | deutsch | español | Example Varieties Exemples Beispielssorten Variedades ejemplo | Note/ Nota |
|------------|--|--|---|--|--|---------------|
| 53. | <u>QL</u> <u>VG</u> | <u>(+)</u> | | | | |
| | <u>Resistance to <i>Bremia lactuca</i> (BI)</u> <u>Isolate BI: 36EU</u> | <u>Résistance à <i>Bremia lactuca</i> (BI)</u> <u>Isolat BI: 36EU</u> | <u>Resistenz gegen <i>Bremia lactuca</i> (BI)</u> <u>Isolat BI: 36EU</u> | <u>Resistencia a <i>Bremia lactuca</i> (BI)</u> <u>Aislado BI: 36EU</u> | | |
| | absent | absente | fehlend | ausente | <u>Bartoli, RYZ2164</u> | <u>1</u> |
| | <u>present</u> | <u>présente</u> | <u>vorhanden</u> | <u>presente</u> | <u>Design, Kibrille</u> | <u>9</u> |

- It is proposed to include the new characteristic 53 in Chapter 10 “Technical questionnaire”, Section 5 “Characteristics of the variety to be indicated”.

¹ organized by electronic means, from April 18 to 22, 2022

Proposed revision of explanation Ad. 38 to 52 “Resistance to *Bremia lactucae* (BI)”*Current wording*Ad. 38 to 52: Resistance to *Bremia lactucae* (BI), several isolates

| | | |
|------|--------------------------------|---|
| 1. | Pathogen | <i>Bremia lactucae</i> |
| 2. | Quarantine status | no |
| 3. | Host species | lettuce - <i>Lactuca sativa</i> L. |
| 4. | Source of inoculum | GEVES ² (FR) or Naktuinbouw ³ (NL) |
| 5. | Isolate | BI: 16EU, 17EU, 20-27EU, 29-31EU, 33EU, 35EU |
| 6. | Establishment isolate identity | test on differentials (see table below) |
| 7. | Establishment pathogenicity | test on susceptible varieties |
| 8. | Multiplication inoculum | |
| 8.2 | Multiplication variety | susceptible variety, for example Green Towers. For higher isolates, a variety with defeated resistance may be preferable to keep the isolate fit. |
| 8.8 | Shelflife/viability inoculum | 2 hours at room temperature; 2 days in fridge |
| 9. | Format of the test | |
| 9.1 | Number of plants per genotype | at least 20 |
| 9.2 | Number of replicates | - |
| 9.3 | Control varieties | (informative) differentials (see table below) |
| 9.4 | Test design | - |
| 9.5 | Test facility | climate room |
| 9.6 | Temperature | 15°C-18°C |
| 9.7 | Light | adequate for good plant growth; seedlings should not etiolate. option: reduced light 24 hours after inoculation |
| 9.8 | Season | - |
| 9.9 | Special measures | plants may grow on wet blotting paper with or without a nutrient solution, on sand or on potting soil (see point 13). high humidity (>90%) is essential for infection and sporulation. |
| 10. | Inoculation | |
| 10.1 | Preparation inoculum | washing off from leaves by vigorous shaking in a closed container |
| 10.2 | Quantification inoculum | counting spores; spore density should be 3×10^4 - 1×10^5 |
| 10.3 | Plant stage at inoculation | cotyledon stage |
| 10.4 | Inoculation method | spraying till run-off. option: reduced light 24 hours after inoculation |
| 10.5 | First observation | beginning of sporulation on susceptible varieties (around 7 days after inoculation) |
| 10.6 | Second observation | 3-4 days after first observation (around 10 days after inoculation) |
| 10.7 | Final observations | 14 days after inoculation two of these three observations may be sufficient, the third notation is optional for observation of evolution of symptoms in case of doubt. the day of maximum sporulation should occur in this period. |

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| | | |
|------|---|--|
| 11. | Observations | |
| 11.1 | Method | visual observation of sporulation and necrotic reaction to infection |
| 11.2 | Observation scale | <p>resistant:</p> <p>class 0 no sporulation, no necrosis</p> <p>class 1 no sporulation, necrosis present</p> <p>class 2 weak sporulation (much less than susceptible control) with necrosis</p> <p>class 3 weak sporulation (less than susceptible control and not evolving between second and third observation) with necrosis</p> <p>class 4 very sparse sporulation (not evolving between second and third observation) without necrosis</p> <p>susceptible:</p> <p>class 5 reduced sporulation (compared to susceptible control) without necrosis</p> <p>class 6 normal sporulation without necrosis</p> |
| 11.3 | Validation of test | <p>on standards</p> <p>in case of normal sporulation (same level as susceptible control) with necrosis another test on bigger plants or other substrate must be undertaken.</p> |
| 12. | Interpretation of data in terms of UPOV characteristic states | <p>class 0, 1, 2, 3 and 4: resistant</p> <p>class 5 and 6: susceptible</p> |
| 13. | Critical control points | <p>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.</p> <p>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.</p> <p>in case of use of nutritive solution on blotting paper, a fungicide can be added to avoid contamination by saprophytes.</p> |

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/>. Pictures for the observation scale are also provided.

| Isolates | Differentials | GreenTowers | Dandie | R4T57D | UC Dm14 | NunDm15 | CGDm16 | Colorado | FRsal-1 | Argelés | RYZ 2164 | RYZ910457 | Bedford | Balesta | Bartoli | Design | Kitbrille |
|----------|---------------|-------------|--------|--------|---------|---------|--------|----------|---------|---------|----------|-----------|---------|---------|---------|--------|-----------|
| BI: 16EU | + | + | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| BI: 17EU | + | + | - | + | + | - | + | + | - | - | - | (+) | - | - | - | - | - |
| BI: 20EU | + | + | + | - | - | + | + | - | - | - | - | - | - | - | - | - | - |
| BI: 21EU | + | + | + | - | + | + | - | + | - | - | - | - | - | - | - | - | - |
| BI: 22EU | + | - | + | + | + | - | + | - | - | - | - | - | + | - | - | - | - |
| BI: 23EU | + | + | + | - | - | + | - | - | + | - | - | - | - | - | - | - | - |
| BI: 24EU | + | - | + | - | - | + | + | - | + | - | - | - | - | - | - | (-) | - |
| BI: 25EU | + | - | + | - | - | + | + | + | - | - | - | - | - | - | - | - | - |
| BI: 26EU | + | + | + | - | - | + | + | + | + | - | - | - | - | - | - | - | - |
| BI: 27EU | + | + | + | + | + | - | + | - | + | + | - | (-) | + | - | - | - | - |
| BI: 29EU | + | - | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| BI: 30EU | + | - | + | + | + | - | + | - | + | + | - | - | - | - | + | - | - |
| BI: 31EU | + | + | + | + | - | - | + | - | - | + | + | - | - | - | + | - | - |
| BI: 33EU | + | - | + | + | + | + | + | + | + | + | - | - | - | - | + | + | + |
| BI: 35EU | + | - | + | + | + | + | + | + | + | + | + | - | - | - | + | + | + |

*Proposed new wording*Ad. 38 to ~~52-53~~: Resistance to *Bremia lactucae* (Bl), several isolates

| | | |
|------|--------------------------------|---|
| 1. | Pathogen | <i>Bremia lactucae</i> |
| 2. | Quarantine status | no |
| 3. | Host species | lettuce - <i>Lactuca sativa</i> L. |
| 4. | Source of inoculum | GEVES ⁴ (FR) or Naktuinbouw ⁵ (NL) |
| 5. | Isolate | Bl: 16EU, 17EU, 20-27EU, 29-31EU, 33EU, 35EU, <u>36EU</u> |
| 6. | Establishment isolate identity | test on differentials (see table below) |
| 7. | Establishment pathogenicity | test on susceptible varieties |
| 8. | Multiplication inoculum | |
| 8.2 | Multiplication variety | susceptible variety, for example Green Towers. For higher isolates, For isolates with a higher number, a variety with defeated resistance may be preferable to keep the isolate fit. |
| 8.8 | Shelflife/viability inoculum | 2 hours at room temperature; 2 days in fridge |
| 9. | Format of the test | |
| 9.1 | Number of plants per genotype | at least 20 |
| 9.2 | Number of replicates | - |
| 9.3 | Control varieties | (informative) differentials (see table below) |
| 9.4 | Test design | - |
| 9.5 | Test facility | climate room |
| 9.6 | Temperature | 15°C-18°C |
| 9.7 | Light | adequate for good plant growth; seedlings should not etiolate. option: reduced light 24 hours after inoculation |
| 9.8 | Season | - |
| 9.9 | Special measures | plants may grow on wet blotting paper with or without a nutrient solution, on sand or on potting soil (see point 13). high humidity (>90%) is essential for infection and sporulation. |
| 10. | Inoculation | |
| 10.1 | Preparation inoculum | washing off from leaves by vigorous shaking in a closed container |
| 10.2 | Quantification inoculum | counting spores; spore density should be 3×10^4 - 1×10^5 |
| 10.3 | Plant stage at inoculation | cotyledon stage |
| 10.4 | Inoculation method | spraying till run-off. option: reduced light 24 hours after inoculation |
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| 10.7 | Final observations | 14 days after inoculation two of these three observations may be sufficient, the third notation is optional for observation of evolution of symptoms in case of doubt. the day of maximum sporulation should occur in this period. |

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| | | |
|------|---|--|
| 11. | Observations | |
| 11.1 | Method | visual observation of sporulation and necrotic reaction to infection |
| 11.2 | Observation scale | <p>resistant:</p> <p>class 0 no sporulation, no necrosis</p> <p>class 1 no sporulation, necrosis present</p> <p>class 2 weak sporulation (much less than susceptible control) with necrosis</p> <p>class 3 weak sporulation (less than susceptible control and not evolving between second and third observation) with necrosis</p> <p>class 4 very sparse sporulation (not evolving between second and third observation) without necrosis</p> <p>susceptible:</p> <p>class 5 reduced sporulation (compared to susceptible control) without necrosis</p> <p>class 6 normal sporulation without necrosis</p> |
| 11.3 | Validation of test | <p>on standards</p> <p>in case of normal sporulation (same level as susceptible control) with necrosis another test on bigger plants or other substrate must be undertaken.</p> |
| 12. | Interpretation of data in terms of UPOV characteristic states | <p>class 0, 1, 2, 3 and 4: resistant</p> <p>class 5 and 6: susceptible</p> |
| 13. | Critical control points | <p>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.</p> <p>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.</p> <p>in case of use of nutritive solution on blotting paper, a fungicide can be added to avoid contamination by saprophytes.</p> |

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/>. Pictures for the observation scale are also provided.

| Isolates | Differentials | Green Towers | Dandle | R4T57D | UC Dm14 | NunDm15 | CGDm16 | Colorado | F rRsal-1 | Argelés | RYZ 2164 | RYZ910457 | Bedford | Balesta | Bartoli | Design | Kibrille |
|----------|---------------|--------------|--------|--------|---------|---------|--------|----------|-----------|---------|----------|-----------|---------|---------|---------|--------|----------|
| BI: 16EU | + | + | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| BI: 17EU | + | + | - | + | + | - | + | + | - | - | (+) | - | - | - | - | - | - |
| BI: 20EU | + | + | + | - | - | + | + | - | - | - | - | - | - | - | - | - | - |
| BI: 21EU | + | + | + | - | + | + | - | + | - | - | - | - | - | - | - | - | - |
| BI: 22EU | + | - | + | + | + | - | + | - | - | - | - | - | + | - | - | - | - |
| BI: 23EU | + | + | + | - | - | + | - | - | + | - | - | - | - | - | - | - | - |
| BI: 24EU | + | - | + | - | - | + | + | - | + | - | - | - | - | - | - | (-) | - |
| BI: 25EU | + | - | + | - | - | + | + | + | - | - | - | - | - | - | - | - | - |
| BI: 26EU | + | + | + | - | - | + | + | + | + | - | - | - | - | - | - | - | - |
| BI: 27EU | + | + | + | + | + | - | + | - | + | + | (-) | + | - | - | - | - | - |
| BI: 29EU | + | - | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| BI: 30EU | + | - | + | + | + | - | + | - | + | + | - | - | - | - | - | + | - |
| BI: 31EU | + | + | + | + | - | - | + | - | - | + | + | - | - | - | - | + | - |
| BI: 33EU | + | - | + | + | + | + | + | + | + | + | - | - | - | - | - | + | + |
| BI: 35EU | + | - | + | + | + | + | + | + | + | + | + | - | - | - | - | + | + |
| BI: 36EU | + | + | + | + | - | + | + | + | + | + | + | - | - | + | - | - | - |

Resistance is indicated with – or (-), susceptibility is indicated with + or (+). The brackets indicate a lower and sometimes variable level of expression of the phenotype.

Proposed inclusion of characteristics from the Table of Characteristics in the Technical Questionnaire

6. The TWV is invited to consider the inclusion of the following characteristics in the TQ (characteristics for inclusion indicated in highlight and underline):

| Char. No. | (*) | Characteristic Name | Comments |
|-----------|-----|--|--|
| 1 | (*) | Seed: color | |
| 2 | (*) | <u>Plant: diameter</u> | |
| 3 | (*) | <u>Plant: degree of overlapping of upper part of leaves</u> | |
| 6 | (*) | <u>Leaf: number of divisions</u> | |
| 7 | | <u>Only varieties with Leaf: number of divisions: absent or very few: Leaf: shape</u> | Option "not applicable" added |
| 11 | (*) | Leaf: anthocyanin coloration | |
| 12 | (*) | <u>Leaf: hue of anthocyanin coloration</u> | Option "not applicable" added (see Char. 11) |
| 13 | | <u>Leaf: area covered by anthocyanin coloration</u> | Option "not applicable" added (see Char. 11) |
| 14 | (*) | <u>Leaf: color</u> | |
| 15 | (*) | Leaf: intensity of green color | |
| 18 | (*) | <u>Leaf: blistering</u> | |
| 20 | (*) | <u>Leaf: undulation of margin</u> | |
| 22 | | <u>Leaf: depth of incisions of margin</u> | |
| 25 | (*) | <u>Leaf: venation</u> | |
| 26 | | <u>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Head: size</u> | Option "not applicable" added |
| 27 | (*) | <u>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Head: shape in longitudinal section</u> | Option "not applicable" added |
| 34 | | <u>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Time of harvest maturity</u> | Option "not applicable" added |
| 35 | (*) | Time of beginning of bolting | |
| 38 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 16EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 39 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 17EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 40 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 20EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 41 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 21EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 42 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 22EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 43 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 23EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 44 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 24EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 45 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 25EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 46 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 26EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 47 | | <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 27EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |

| | | |
|----|--|--|
| 48 | <u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 29EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 49 | <u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 30EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 50 | <u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 31EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 51 | <u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 33EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 52 | <u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 35EU</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 53 | <u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 36EU</u> | Proposed addition based on current partial revision |
| 54 | <u>Resistance to Lettuce mosaic virus (LMV) Pathotype II</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 55 | <u>Resistance to <i>Nasonovia ribisnigri</i> (Nr) Biotype Nr: 0</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |
| 56 | <u>Resistance to <i>Fusarium oxysporum</i> f. sp. <i>lactucae</i> (Fol) Race 1</u> | Currently in TQ 7 with option "Not tested"; "Not tested" added to TQ 5 |

| | | |
|-------------------------|-----------------|-------------------|
| 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 (2) Plant: diameter | | |
| <u>very small</u> | <u>Tom Thumb</u> | <u>1 []</u> |
| <u>very small to small</u> | | <u>2 []</u> |
| <u>small</u> | <u>Gotte à graine blanche</u> | <u>3 []</u> |
| <u>small to medium</u> | | <u>4 []</u> |
| <u>medium</u> | <u>Clarion, Verpia</u> | <u>5 []</u> |
| <u>medium to large</u> | | <u>6 []</u> |
| <u>large</u> | <u>Great Lakes 659</u> | <u>7 []</u> |
| <u>large to very large</u> | | <u>8 []</u> |
| <u>very large</u> | <u>El Toro</u> | <u>9 []</u> |
| 5.3 (3) Plant: degree of overlapping of upper part of leaves | | |
| <u>absent or weak</u> | <u>Actarus, Aquarel,</u> <u>Blonde à couper améliorée, Curtis,</u> <u>Lollo rossa</u> | <u>1 []</u> |
| <u>medium</u> | <u>Augusta, Clarion, Fiorella</u> | <u>2 []</u> |
| <u>strong</u> | <u>Roxette, Vanguard 75</u> | <u>3 []</u> |

| TECHNICAL QUESTIONNAIRE | | Page {x} of {y} | Reference Number: |
|-------------------------|---|-----------------|-------------------|
| Characteristics | Example Varieties | Note | |
| 5.4 | Leaf: number of divisions | | |
| (6) | | | |
| absent or very few | Fiorella, Lollo rossa | 1 [] | |
| very few to few | | 2 [] | |
| few | Curletta, Rodagio | 3 [] | |
| few to medium | | 4 [] | |
| medium | Ezabel, Jadigon | 5 [] | |
| medium to many | | 6 [] | |
| many | Expedition, Multired 54 | 7 [] | |
| many to very many | | 8 [] | |
| very many | Excite, Ezfrill, Telex | 9 [] | |
| 5.5 | Only varieties with Leaf: number of divisions: absent or very few: Leaf: shape | | |
| (7) | | | |
| 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 [] | |
| not applicable | | [] | |
| 5.2 6 | Leaf: anthocyanin coloration | | |
| (11) | | | |
| absent or very weak | Clarion | 1 [] | |
| very weak to weak | | 2 [] | |
| weak | Du bon jardinier | 3 [] | |
| weak to medium | | 4 [] | |
| medium | Lollo rossa, Luana | 5 [] | |
| medium to strong | | 6 [] | |
| strong | Merveille des quatre saisons | 7 [] | |
| strong to very strong | | 8 [] | |
| very strong | Iride, Revolution | 9 [] | |

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

| Characteristics | Example Varieties | Note |
|---|--|-------|
| 5.7 (12) Leaf: hue of anthocyanin coloration | | |
| <u>reddish</u> | <u>Lollo rossa</u> | 1 [] |
| <u>purplish</u> | <u>Iride</u> | 2 [] |
| <u>brownish</u> | <u>Luana, Maravilla de Verano</u> | 3 [] |
| <u>not applicable</u> | | [] |
| 5.8 (13) Leaf: area covered by anthocyanin coloration | | |
| <u>very small</u> | <u>Steirer Krauthauptel</u> | 1 [] |
| <u>very small to small</u> | | 2 [] |
| <u>small</u> | <u>Diablo</u> | 3 [] |
| <u>small to medium</u> | | 4 [] |
| <u>medium</u> | <u>Luana</u> | 5 [] |
| <u>medium to large</u> | | 6 [] |
| <u>large</u> | <u>Merveille des quatre saisons</u> | 7 [] |
| <u>large to very large</u> | | 8 [] |
| <u>very large</u> | <u>Bijou, Revolution</u> | 9 [] |
| <u>not applicable</u> | | [] |
| 5.9 (14) Leaf: color | | |
| <u>green</u> | <u>Verpia</u> | 1 [] |
| <u>yellowish green</u> | <u>Dorée de printemps</u> | 2 [] |
| <u>greyish green</u> | <u>Celtuce, Du bon jardinier</u> | 3 [] |
| 5.3 10 (15) Leaf: intensity of green color | | |
| <u>very light</u> | | 1 [] |
| <u>very light to light</u> | | 2 [] |
| <u>light</u> | <u>Blonde maraîchère, Lollo Bionda</u> | 3 [] |
| <u>light to medium</u> | | 4 [] |
| <u>medium</u> | <u>Aquarel, Clarion</u> | 5 [] |
| <u>medium to dark</u> | | 6 [] |
| <u>dark</u> | <u>Expedition, Verpia</u> | 7 [] |
| <u>dark to very dark</u> | | 8 [] |
| <u>very dark</u> | <u>Pascal, Verdatrix</u> | 9 [] |

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| TECHNICAL QUESTIONNAIRE | Page {x} of {y} | Reference Number: |
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| Characteristics | Example Varieties | Note |
|--|---|-------|
| 5.11 (18) <u>Leaf: blistering</u> | | |
| <u>absent or very weak</u> | <u>Duplex, Sartre</u> | 1 [] |
| <u>very weak to weak</u> | | 2 [] |
| <u>weak</u> | <u>Fiorella</u> | 3 [] |
| <u>weak to medium</u> | | 4 [] |
| <u>medium</u> | <u>Commodore</u> | 5 [] |
| <u>medium to strong</u> | | 6 [] |
| <u>strong</u> | <u>Blonde de Paris, Xanadu</u> | 7 [] |
| <u>strong to very strong</u> | | 8 [] |
| <u>very strong</u> | <u>Blonde de Doulon, Iride, Karioka</u> | 9 [] |
| 5.12 (20) <u>Leaf: undulation of margin</u> | | |
| <u>absent or very weak</u> | <u>Tiago</u> | 1 [] |
| <u>very weak to weak</u> | | 2 [] |
| <u>weak</u> | <u>Commodore</u> | 3 [] |
| <u>weak to medium</u> | | 4 [] |
| <u>medium</u> | <u>Noisette, Pentared</u> | 5 [] |
| <u>medium to strong</u> | | 6 [] |
| <u>strong</u> | <u>Calmar, Invicta</u> | 7 [] |
| <u>strong to very strong</u> | | 8 [] |
| <u>very strong</u> | <u>Lollo rossa</u> | 9 [] |
| 5.13 (22) <u>Leaf: depth of incisions of margin</u> | | |
| <u>absent or very shallow</u> | <u>Actarus, Clarion, Tiago</u> | 1 [] |
| <u>very shallow to shallow</u> | | 2 [] |
| <u>shallow</u> | <u>Pentared, Unicum</u> | 3 [] |
| <u>shallow to medium</u> | | 4 [] |
| <u>medium</u> | <u>Santarinas</u> | 5 [] |
| <u>medium to deep</u> | | 6 [] |
| <u>deep</u> | <u>Expedition</u> | 7 [] |
| <u>deep to very deep</u> | | 8 [] |
| <u>very deep</u> | | 9 [] |
| 5.14 (25) <u>Leaf: venation</u> | | |
| <u>not flabellate</u> | <u>Verpia, Xanadu</u> | 1 [] |
| <u>semi-flabellate</u> | <u>Kibrille, Murai</u> | 2 [] |
| <u>flabellate</u> | <u>Locarno, Roxette</u> | 3 [] |

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| TECHNICAL QUESTIONNAIRE | Page {x} of {y} | Reference Number: |
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| Characteristics | Example Varieties | Note |
|---|-----------------------------------|-------|
| 5.15 (26) <u>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Head: size</u> | | |
| <u>very small</u> | <u>Tom Thumb</u> | 1 [] |
| <u>very small to small</u> | | 2 [] |
| <u>small</u> | <u>Xanadu</u> | 3 [] |
| <u>small to medium</u> | | 4 [] |
| <u>medium</u> | <u>Fiorella, Soraya</u> | 5 [] |
| <u>medium to large</u> | | 6 [] |
| <u>large</u> | <u>Great Lakes 659</u> | 7 [] |
| <u>large to very large</u> | | 8 [] |
| <u>very large</u> | <u>Blonde maraichère, El Toro</u> | 9 [] |
| <u>not applicable</u> | | [] |
| 5.16 (27) <u>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Head: shape in longitudinal section</u> | | |
| <u>narrow elliptic</u> | <u>Verte maraichère</u> | 1 [] |
| <u>broad elliptic</u> | <u>Amadeus, Sucrine</u> | 2 [] |
| <u>circular</u> | <u>Verpia</u> | 3 [] |
| <u>narrow oblate</u> | <u>Ametist</u> | 4 [] |
| <u>not applicable</u> | | [] |
| 5.17 (34) <u>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Time of harvest maturity</u> | | |
| <u>very early</u> | <u>Gotte jaune d'or</u> | 1 [] |
| <u>very early to early</u> | | 2 [] |
| <u>early</u> | <u>Pantlika, Sucrine</u> | 3 [] |
| <u>early to medium</u> | | 4 [] |
| <u>medium</u> | <u>Clarion</u> | 5 [] |
| <u>medium to late</u> | | 6 [] |
| <u>late</u> | <u>Blonde maraichère, Calmar</u> | 7 [] |
| <u>late to very late</u> | | 8 [] |
| <u>very late</u> | <u>El Toro, Pinokkio</u> | 9 [] |
| <u>not applicable</u> | | [] |

| TECHNICAL QUESTIONNAIRE | Page {x} of {y} | Reference Number: |
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| Characteristics | Example Varieties | Note |
| 5.4 18 Time of beginning of bolting (35) | | |
| very early | Blonde à couper améliorée | 1 [] |
| very early to early | | 2 [] |
| early | Gotte à graine blanche | 3 [] |
| early to medium | | 4 [] |
| medium | Pantlika | 5 [] |
| medium to late | | 6 [] |
| late | Hilde II | 7 [] |
| late to very late | | 8 [] |
| very late | Erika, Roxette | 9 [] |
| 5.19 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 16EU (38) | | |
| <u>absent</u> | <u>Green Towers</u> | 1 [] |
| <u>present</u> | <u>Argelès</u> | 9 [] |
| <u>not tested</u> | | [] |
| 5.20 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 17EU (39) | | |
| <u>absent</u> | <u>Green Towers</u> | 1 [] |
| <u>present</u> | <u>Argelès</u> | 9 [] |
| <u>not tested</u> | | [] |
| 5.21 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 20EU (40) | | |
| <u>absent</u> | <u>Green Towers</u> | 1 [] |
| <u>present</u> | <u>FrRsal-1</u> | 9 [] |
| <u>not tested</u> | | [] |
| 5.22 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 21EU (41) | | |
| <u>absent</u> | <u>Green Towers</u> | 1 [] |
| <u>present</u> | <u>Argelès, Colorado</u> | 9 [] |
| <u>not tested</u> | | [] |
| 5.23 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 22EU (42) | | |
| <u>absent</u> | <u>Green Towers</u> | 1 [] |
| <u>present</u> | <u>FrRsal-1</u> | 9 [] |
| <u>not tested</u> | | [] |
| 5.24 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 23EU (43) | | |
| <u>absent</u> | <u>Green Towers</u> | 1 [] |
| <u>present</u> | <u>Colorado</u> | 9 [] |
| <u>not tested</u> | | [] |

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| TECHNICAL QUESTIONNAIRE | Page {x} of {y} | Reference Number: |
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| Characteristics | Example Varieties | Note |
|---|--------------------------------|--------------|
| <u>5.25</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 24EU</u> <u>(44)</u> | | |
| <u>absent</u> | <u>Argelès, Colorado</u> | <u>1 []</u> |
| <u>present</u> | <u>Dandie, NunDm15, UCDm14</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.26</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 25EU</u> <u>(45)</u> | | |
| <u>absent</u> | <u>Colorado</u> | <u>1 []</u> |
| <u>present</u> | <u>Argelès</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.27</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 26EU</u> <u>(46)</u> | | |
| <u>absent</u> | <u>Colorado</u> | <u>1 []</u> |
| <u>present</u> | <u>Balesta, Bedford</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.28</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 27EU</u> <u>(47)</u> | | |
| <u>absent</u> | <u>Balesta, Colorado</u> | <u>1 []</u> |
| <u>present</u> | <u>FrRsal-1</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.29</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 29EU</u> <u>(48)</u> | | |
| <u>absent</u> | <u>Argelès</u> | <u>1 []</u> |
| <u>present</u> | <u>Balesta</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.30</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 30EU</u> <u>(49)</u> | | |
| <u>absent</u> | <u>Argelès, Colorado</u> | <u>1 []</u> |
| <u>present</u> | <u>Balesta</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.31</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 31EU</u> <u>(50)</u> | | |
| <u>absent</u> | <u>Colorado, RYZ910457</u> | <u>1 []</u> |
| <u>present</u> | <u>Argelès, Balesta</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.32</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 33EU</u> <u>(51)</u> | | |
| <u>absent</u> | <u>Kibrille, RYZ2164</u> | <u>1 []</u> |
| <u>present</u> | <u>RYZ910457</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |

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| Characteristics | Example Varieties | Note |
|---|---|--------------|
| <u>5.33</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 35EU</u> <u>(52)</u> | | |
| <u>absent</u> | <u>Design, Kibrille</u> | <u>1 []</u> |
| <u>present</u> | <u>Bartoli</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.34</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 36EU</u> <u>(53)</u> | | |
| <u>absent</u> | <u>Bartoli, RYZ2164</u> | <u>1 []</u> |
| <u>present</u> | <u>Design, Kibrille</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.35</u> <u>Resistance to <i>Lettuce mosaic virus</i> (LMV) Pathotype II</u> <u>(53)</u> | | |
| <u>absent</u> | <u>Bijou, Hilde II, Sprinter, Sucrine</u> | <u>1 []</u> |
| <u>present</u> | <u>Capitan, Corsica</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.36</u> <u>Resistance to <i>Nasonovia ribisnigri</i> (Nr) Biotype Nr: 0</u> <u>(54)</u> | | |
| <u>absent</u> | <u>Abel, Green Towers, Nadine</u> | <u>1 []</u> |
| <u>present</u> | <u>Barcelona, Bedford, Dynamite, Silvinas</u> | <u>9 []</u> |
| <u>not tested</u> | | <u>[]</u> |
| <u>5.36</u> <u>Resistance to <i>Fusarium oxysporum</i> f.sp. <i>lactucae</i> (Fol) Race 1</u> <u>(55)</u> | | |
| <u>susceptible</u> | <u>Cobham Green, Patriot</u> | <u>1 []</u> |
| <u>moderately resistant</u> | <u>Affic, Fuzila, Natexis</u> | <u>2 []</u> |
| <u>highly resistant</u> | <u>Costa Rica No. 4, Romasol</u> | <u>3 []</u> |
| <u>not tested</u> | | <u>[]</u> |

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