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| International Union for the Protection of New Varieties of Plants |  |

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| Technical Working Party for VegetablesFifty-Second SessionBeijing, China, September 17 to 21, 2018 | TWV/52/4Original: EnglishDate: August 22, 2018 |

Partial revision of the Test Guidelines for LETTUCE

Document prepared 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).

 The Technical Working Party for Vegetables (TWV), at its fift-first session, held in Roelofarendsveen, Netherlands, from July 3 to 7, 2017, agreed that the Test Guidelines for lettuce (document TG/13/11) be partially revised for new *Bremia lactucae* races and adaptation of *Bremia lactucae* race names (see document TWV/51/16 “Report”, Annex IV).

 The following changes are proposed:

1. Revision of Characteristics 38 to 50: addition of “EU” to the isolate code;
2. Addition of “Resistance to Bremia lactucae (Bl) Isolate Bl: 33EU” and “Resistance to Bremia lactucae (Bl) Isolate Bl: 35EU” including example varieties;
3. Revision of explanation Ad. 38 to 50 in Chapter 8.2 “Explanations for individual characteristics”;
4. Addition of “Resistance to Bremia lactucae (Bl) Isolate Bl: 33EU” and “Resistance to Bremia lactucae (Bl) Isolate Bl: 35EU” to Chapter TQ 7.3 “Other information”;

 The proposed changes are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

Proposal to revise Characteristics 38 to 50: addition of “EU” to the isolate code

|  |  | English | français | deutsch | español | Example VarietiesExemplesBeispielssortenVariedades ejemplo | Note/Nota |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **38.** |  | **QL** | **VG** | **(+)** |  |  |
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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 16EU** |

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| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 16EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 16EU** |

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| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 16EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Green Towers | 1 |
|  |  | present | présente | vorhanden | presente | Argelès | 9 |
| **39.** |  | **QL** | **VG** | **(+)** |  |  |
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| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 17EU** |

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| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 17EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 17EU** |

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| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 17EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Green Towers | 1 |
|  |  | present | présente | vorhanden | presente | Argelès | 9 |
| **40.** |  | **QL** | **VG** | **(+)** |  |  |
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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 20EU** |

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| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 20EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 20EU** |

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| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 20EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Green Towers | 1 |
|  |  | present | présente | vorhanden | presente | FrRsal-1 | 9 |
| **41.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 21EU** |

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| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 21EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 21EU** |

 |

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| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 21EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Green Towers | 1 |
|  |  | present | présente | vorhanden | presente | Argelès, Colorado | 9 |
| **42.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 22EU** |

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|  |
| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 22EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 22EU** |

 |

|  |
| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 22EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Green Towers | 1 |
|  |  | present | présente | vorhanden | presente | FrRsal-1 | 9 |
| **43.** |  | **QL** | **VG** | **(+)** |  |  |
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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 23EU** |

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| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 23EU** |

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|  |
| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 23EU** |

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|  |
| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 23EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Green Towers | 1 |
|  |  | present | présente | vorhanden | presente | Colorado | 9 |
| **44.** |  | **QL** | **VG** | **(+)** |  |  |
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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 24EU** |

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| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 24EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 24EU** |

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| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 24EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Argelès, Colorado | 1 |
|  |  | present | présente | vorhanden | presente | Dandie, NunDm15, UCDm14 | 9 |

|  |  | English | français | deutsch | español | Example VarietiesExemplesBeispielssortenVariedades ejemplo | Note/Nota |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **45.** |  | **QL** | **VG** | **(+)** |  |  |
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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 25EU** |

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| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 25EU** |

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| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 25EU** |

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| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 25EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Colorado | 1 |
|  |  | present | présente | vorhanden | presente | Argelès | 9 |
| **46.** |  | **QL** | **VG** | **(+)** |  |  |
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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 26EU** |

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| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 26EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 26EU** |

 |

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| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 26EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Colorado | 1 |
|  |  | present | présente | vorhanden | presente | Balesta, Bedford | 9 |
| **47.** |  | **QL** | **VG** | **(+)** |  |  |
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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 27EU** |

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| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 27EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 27EU** |

 |

|  |
| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 27EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Balesta, Colorado | 1 |
|  |  | present | présente | vorhanden | presente | FrRsal-1 | 9 |
| **48.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 29EU** |

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| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 29EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 29EU** |

 |

|  |
| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 29EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Argelès | 1 |
|  |  | present | présente | vorhanden | presente | Balesta | 9 |
| **49.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 30EU** |

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| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 30EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 30EU** |

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| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 30EU** |

 |  |  |
|  |  | absent | absent | fehlend | ausente | Argelès, Colorado | 1 |
|  |  | present | present | vorhanden | presente | Balesta | 9 |
| **50.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 31EU** |

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| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 31EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 31EU** |

 |

|  |
| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 31EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Colorado, RYZ910457 | 1 |
|  |  | present | présente | vorhanden | presente | Argelès, Balesta | 9 |

## Proposal to add “Resistance to Bremia lactucae (Bl) Isolate Bl: 33EU” and “Resistance to Bremia lactucae (Bl) Isolate Bl: 35EU” including example varieties

*Current wording:*

|  |  | English | français | deutsch | español | Example VarietiesExemplesBeispielssortenVariedades ejemplo | Note/Nota |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **50.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

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| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 31** |

 |

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| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 31** |

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|  |
| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 31** |

 |

|  |
| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 31** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Colorado, RYZ910457 | 1 |
|  |  | present | présente | vorhanden | presente | Argelès, Balesta | 9 |
| **51.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

|  |
| --- |
| **Resistance to *Lettuce mosaic virus* (LMV) Pathotype II** |

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| --- |
| **Résistance au *Lettuce mosaic virus* (LMV) Pathotype II** |

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| --- |
| **Resistenz gegen *Lettuce mosaic virus* (LMV) Pathotyp II** |

 |

|  |
| --- |
| **Resistencia al *Lettuce mosaic virus* (LMV), Patotipo II** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Bijou, Hilde II, Sprinter, Sucrine | 1 |
|  |  | present | présente | vorhanden | presente | Capitan, Corsica | 9 |
| **52.** |  | **QL** | **MS/VG** | **(+)** |  |  |
|  |  |

|  |
| --- |
| **Resistance to *Nasonovia ribisnigri* (Nr)** **Biotype Nr: 0** |

 |

|  |
| --- |
| **Résistance à *Nasonovia ribisnigri* (Nr)** **Biotype Nr: 0** |

 |

|  |
| --- |
| **Resistenz gegen *Nasonovia ribisnigri* (Nr)** **Biotyp Nr: 0** |

 |

|  |
| --- |
| **Resistencia a *Nasonovia ribisnigri* (Nr)** **Biotipo Nº 0** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Abel, Green Towers, Nadine | 1 |
|  |  | present | présente | vorhanden | presente | Barcelona, Bedford, Dynamite, Silvinas | 9 |
| **53.** |  | **QN** | **MS/VG** | **(+)** |  |  |
|  |  |

|  |
| --- |
| **Resistance to *Fusarium oxysporum* f.sp. *lactucae* (Fol) Race 1** |

 |

|  |
| --- |
| **Résistance à *Fusarium oxysporum* f.sp. *lactucae* (Fol)** **Race 1** |

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|  |
| --- |
| **Resistenz gegen *Fusarium oxysporum* f.sp. *lactucae* (Fol) Pathotyp 1** |

 |

|  |
| --- |
| **Resistencia a *Fusarium oxysporum* f.sp. *lactucae* (Fol) Raza 1** |

 |  |  |
|  |  | susceptible | sensible | anfällig | susceptible | Cobham Green, Patriot | 1 |
|  |  | moderately resistant | modérément résistante | mäßig resistent | moderadamente resistente | Affic, Fuzila, Natexis | 2 |
|  |  | highly resistant | hautement résistante | hochresistent | muy resistente | Costa Rica No. 4, Romasol | 3 |

*Proposed new wording*

|  |  | English | français | deutsch | español | Example VarietiesExemplesBeispielssortenVariedades ejemplo | Note/Nota |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **50.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

|  |
| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 31** |

 |

|  |
| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 31** |

 |

|  |
| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 31** |

 |

|  |
| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 31** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Colorado, RYZ910457 | 1 |
|  |  | present | présente | vorhanden | presente | Argelès, Balesta | 9 |
| **51.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

|  |
| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 33EU** |

 |

|  |
| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 33EU** |

 |

|  |
| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 33EU** |

 |

|  |
| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 33EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Kibrille, RYZ2164 | 1 |
|  |  | present | présente | vorhanden | presente | RYZ910457 | 9 |
|  |  |  |  |  |  |  |  |
| **52.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

|  |
| --- |
| **Resistance to *Bremia lactucae*(Bl) Isolate Bl: 35EU** |

 |

|  |
| --- |
| **Résistance à *Bremia lactucae* (Bl) Isolat Bl: 35EU** |

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| --- |
| **Resistenz gegen *Bremia lactucae* (Bl) Isolat Bl: 35EU** |

 |

|  |
| --- |
| **Resistencia a *Bremia lactucae* (Bl) Aislado Bl: 35EU** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Design, Kibrille | 1 |
|  |  | present | présente | vorhanden | presente | Bartoli | 9 |
| **~~51.~~53.** |  | **QL** | **VG** | **(+)** |  |  |
|  |  |

|  |
| --- |
| **Resistance to *Lettuce mosaic virus* (LMV) Pathotype II** |

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| --- |
| **Résistance au *Lettuce mosaic virus* (LMV) Pathotype II** |

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| --- |
| **Resistenz gegen *Lettuce mosaic virus* (LMV) Pathotyp II** |

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| --- |
| **Resistencia al *Lettuce mosaic virus* (LMV), Patotipo II** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Bijou, Hilde II, Sprinter, Sucrine | 1 |
|  |  | present | présente | vorhanden | presente | Capitan, Corsica | 9 |
| **~~52.~~****54.** |  | **QL** | **MS/VG** | **(+)** |  |  |
|  |  |

|  |
| --- |
| **Resistance to *Nasonovia ribisnigri* (Nr)** **Biotype Nr: 0** |

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| --- |
| **Résistance à *Nasonovia ribisnigri* (Nr)** **Biotype Nr: 0** |

 |

|  |
| --- |
| **Resistenz gegen *Nasonovia ribisnigri* (Nr)** **Biotyp Nr: 0** |

 |

|  |
| --- |
| **Resistencia a *Nasonovia ribisnigri* (Nr)** **Biotipo Nº 0** |

 |  |  |
|  |  | absent | absente | fehlend | ausente | Abel, Green Towers, Nadine | 1 |
|  |  | present | présente | vorhanden | presente | Barcelona, Bedford, Dynamite, Silvinas | 9 |
| **~~53.~~****55.** |  | **QN** | **MS/VG** | **(+)** |  |  |
|  |  |

|  |
| --- |
| **Resistance to *Fusarium oxysporum* f.sp. *lactucae* (Fol) Race 1** |

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| --- |
| **Résistance à *Fusarium oxysporum* f.sp. *lactucae* (Fol)** **Race 1** |

 |

|  |
| --- |
| **Resistenz gegen *Fusarium oxysporum* f.sp. *lactucae* (Fol) Pathotyp 1** |

 |

|  |
| --- |
| **Resistencia a *Fusarium oxysporum* f.sp. *lactucae* (Fol) Raza 1** |

 |  |  |
|  |  | susceptible | sensible | anfällig | susceptible | Cobham Green, Patriot | 1 |
|  |  | moderately resistant | modérément résistante | mäßig resistent | moderadamente resistente | Affic, Fuzila, Natexis | 2 |
|  |  | highly resistant | hautement résistante | hochresistent | muy resistente | Costa Rica No. 4, Romasol | 3 |

## Proposal to revise explanation Ad. 38 to 50 in Chapter 8.2 “Explanations for individual characteristics”

*Current wording*

|  |  |
| --- | --- |
| 1. Pathogen | *Bremia lactucae* |
| 2. Quarantine status | no |
| 3. Host species | lettuce - *Lactuca sativa* L. |
| 4. Source of inoculum | GEVES[[1]](#footnote-2) (FR) or Naktuinbouw[[2]](#footnote-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.for higher isolates, a variety with defeated resistance may be preferable to keep the isolate fit. |
|  8.3 Plant stage at inoculation | cotyledon to first leaf |
|  8.4 Inoculation medium | tap water |
|  8.5 Inoculation method | spraying a spore suspension  |
|  8.6 Harvest of inoculum  | washing off from leaves |
|  8.7 Check of harvested inoculum | counting spores |
|  8.8 Shelf life/viability inoculum | 2 hours at room temperature; 2 days in fridge |
| 9. Format of the test |  |
|  9.1 Number of plants per genotype | at least 20 |
|  9.2 Number of replicates | - |
|  9.3 Control varieties | (informative) differentials (see table below) |
|  9.4 Test design | - |
|  9.5 Test facility | climate room |
|  9.6 Temperature | 15°C-18°C |
|  9.7 Light | adequate for good plant growth; seedlings should not etiolate. option: reduced light 24 hours after inoculation |
|  9.8 Season | - |
|  9.9 Special measures | plants may grow on wet blotting paper with or without a nutrient solution, on sand or on potting soil (see point 13). high humidity (>90%) is essential for infection and sporulation. |
| 10. Inoculation |  |
|  10.1 Preparation inoculum | washing off from leaves by vigorous shaking in a closed container |
|  10.2 Quantification inoculum | counting spores; spore density should be 3.104-1.105 |
|  10.3 Plant stage at inoculation | cotyledon stage |
|  10.4 Inoculation method | spraying till run-off. option: reduced light 24 hours after inoculation |
|  10.5 First observation | beginning of sporulation on susceptible varieties (around 7 days after inoculation) |
|  10.6 Second observation | 3-4 days after first observation (around 10 days after inoculation) |
|  10.7 Final observations | 14 days after inoculation two of these three observations may be sufficient, the third notation is optional for observation of evolution of symptoms in case of doubt. the day of maximum sporulation should occur in this period. |
| 11. Observations |  |
|  11.1 Method | visual observation of sporulation and necrotic reaction to infection |
|  11.2 Observation scale | resistant: |
|  | 0 | no sporulation, no necrosis |
|  | 1 | no sporulation, necrosis present |
|  | 2 | weak sporulation (much less than susceptible control) with necrosis |
|  | 3 | weak sporulation (less than susceptible control and not evolving between second and third observation) with necrosis |
|  | 4 | very sparse sporulation (not evolving between second and third observation) without necrosis |
|  | susceptible: |
|  | 5 | reduced sporulation (compared to susceptible control) without necrosis |
|  | 6 | normal sporulation without necrosis |
|  11.3 Validation of test | on standards in case of normal sporulation (same level as susceptible control) with necrosis another test on bigger plants or other substrate must be undertaken. |
| 12. Interpretation of data in terms of UPOV characteristic states | class 0, 1, 2, 3 and 4: resistantclass 5 and 6: susceptible |
| 13. Critical control points | reaction of standards (the infection pressure may vary between experiments, leading to slight differences in sporulation intensity); when the reactions are not clear the experiment should be repeated.the sowing on soil can be used to see necrosis, but weak sporulation (much less than susceptible control) can appear; when testing on sand, spores can be confused with grains of sand. in case of use of nutritive solution on blotting paper, a fungicide can be added to avoid contamination by saprophytes. |

For reference: The International Bremia Evaluation Board (IBEB) produces regular updates of the host differential reaction table. The most recent table is available through ISF at
<http://www.worldseed.org/our-work/plant-health/other-initiatives/ibeb/>. The table for isolates mentioned in this guideline and illustrations for the observation scale are given.



*Proposed new wording*

Ad. 38 to ~~50~~52: 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[[3]](#footnote-4) (FR) or Naktuinbouw[[4]](#footnote-5) (NL) |
| 5. | Isolate | Bl: 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 |  |
| ~~8.1~~ | ~~Multiplication medium~~ | ~~lettuce plantlets~~ |
| 8.2 | Multiplication variety | susceptible variety, for example Green Towers.for higher isolates, a variety with defeated resistance may be preferable to keep the isolate fit. |
| ~~8.3~~ | ~~Plant stage at inoculation~~ | ~~cotyledon to first leaf~~ |
| ~~8.4~~ | ~~Inoculation medium~~ | ~~tap water~~ |
| ~~8.5~~ | ~~Inoculation method~~ | ~~spraying a spore suspension~~  |
| ~~8.6~~ | ~~Harvest of inoculum~~ | ~~washing off from leaves~~ |
| ~~8.7~~ | ~~Check of harvested inoculum~~ | ~~counting spores~~ |
| 8.8 | 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 3.104-1.105 |
| 10.3 | Plant stage at inoculation | cotyledon stage |
| 10.4 | Inoculation method | spraying till run-off. option: reduced light 24 hours after inoculation |
| 10.5 | First observation | beginning of sporulation on susceptible varieties (around 7 days after inoculation) |
| 10.6 | Second observation | 3-4 days after first observation (around 10 days after inoculation) |
| 10.7 | Final observations | 14 days after inoculation two of these three observations may be sufficient, the third notation is optional for observation of evolution of symptoms in case of doubt. the day of maximum sporulation should occur in this period. |
| 11. | Observations |  |
| 11.1 | Method | visual observation of sporulation and necrotic reaction to infection |
| 11.2 | Observation scale | resistant:class 0 no sporulation, no necrosisclass 1 no sporulation, necrosis presentclass 2 weak sporulation (much less than susceptible control) with necrosisclass 3 weak sporulation (less than susceptible control and not evolving between second and third observation) with necrosisclass 4 very sparse sporulation (not evolving between second and third observation) without necrosissusceptible:class 5 reduced sporulation (compared to susceptible control) without necrosisclass 6 normal sporulation without necrosis |
| 11.3 | Validation of test | on standards in case of normal sporulation (same level as susceptible control) with necrosis another test on bigger plants or other substrate must be undertaken. |
| 12. | Interpretation of data in terms of UPOV characteristic states | class 0, 1, 2, 3 and 4: resistantclass 5 and 6: susceptible |
| 13. | Critical control points | reaction of standards (the infection pressure may vary between experiments, leading to slight differences in sporulation intensity); when the reactions are not clear the experiment should be repeated.the sowing on soil can be used to see necrosis, but weak sporulation (much less than susceptible control) can appear; when testing on sand, spores can be confused with grains of sand. in case of use of nutritive solution on blotting paper, a fungicide can be added to avoid contamination by saprophytes. |

For reference: The International Bremia Evaluation Board (IBEB) produces regular updates of the host differential reaction table. The most recent table is available through ISF at http://www.worldseed.org/our-work/plant-health/other-initiatives/ibeb/. ~~The table for isolates mentioned in this guideline and~~ Also pictures ~~illustrations~~ for the observation scale are given.



## Proposal to add “Resistance to Bremia lactucae (Bl) Isolate Bl: 33EU” and “Resistance to Bremia lactucae (Bl) Isolate Bl: 35EU” to Chapter TQ 7.3 “Other information”

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| #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 informationType (see 5.3 and 8.3 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:[   ](50)         Resistance to *Bremia lactucae* (Bl) Isolate Bl: 31                not tested 0  [   ] absent 1  [   ] present 9  [   ](51)         Resistance to *Bremia lactucae* (Bl) Isolate Bl: 33                not tested 0  [   ] absent 1  [   ] present 9  [   ](52)         Resistance to *Bremia lactucae* (Bl) Isolate Bl: 35                not tested 0  [   ] absent 1  [   ] present 9  [   ](~~51~~ 53) Resistance to Lettuce mosaic virus (LMV) Pathotype II not tested 0  [   ] absent 1  [   ] present 9  [   ](~~52~~ 54) Resistance to Nasonovia ribisnigri (Nr) Biotype Nr: 0 not tested 0  [   ] absent 1  [   ] present 9  [   ](~~53~~ 55)      Resistance to *Fusarium oxysporum* f. sp. *lactucae* (Fol) Race 1            not tested 0  [   ] susceptible 1  [   ] moderately resistant 2  [   ] highly resistant 3 [ ] |
|  |  |   |  |  |

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[End of document]

1. matref@geves.fr [↑](#footnote-ref-2)
2. resistentie@naktuinbouw.nl [↑](#footnote-ref-3)
3. matref@geves.fr [↑](#footnote-ref-4)
4. resistentie@naktuinbouw.nl [↑](#footnote-ref-5)