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UPOV

# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

## TECHNICAL COMMITTEE

## Forty-Seventh Session
## Geneva, April 4 to 6, 2011

PARTIAL REVISIONS OF TEST GUIDELINES

*Document prepared by the Office of the Union*

1. The purpose of this document is to present the proposals for partial revisions to the following Test Guidelines, to be considered by the Technical Committee, at its forty-seventh session, to be held in Geneva from April 4 to 6, 2011:

Test Guidelines for Lettuce (document TG/13/10)

Test Guidelines for Spinach (document TG/55/7)

Test Guidelines for Mandarins (Citrus; Group 1) (document TG/201/1)

PARTIAL REVISION OF THE TEST GUIDELINES FOR LETTUCE (DOCUMENT TG/13/10)

2. The Technical Working Party for Vegetables (TWV) at its forty-fourth session, held in Veliko Tarnovo, Bulgaria, July 5 to 9, 2010, agreed to propose the partial revision of the Test Guidelines for Lettuce (document TG/13/10) as set out in Annexes I to V of this document;

Annex I: Amendment of characteristic 39 in Table of Characteristics

Annex II: Amendment of Ad. 39 in Chapter 8

Annex III: Addition of characteristic 41 “Resistance to *Nasonovia ribisnigri* biotype Nr: 0”

Annex IV: Addition of an explanation in Ad. 41, Chapter 8

Annex V: Addition of item to Chapter 9

PARTIAL REVISION OF THE TEST GUIDELINES FOR SPINACH (DOCUMENT TG/55/7)

3. The Technical Working Party for Vegetables (TWV) at its forty-fourth session, held in Veliko Tarnovo, Bulgaria, July 5 to 9, 2010, agreed to propose to revise Test Guidelines for Spinach TG/55/7 as follows:

(a) Correction of the pathogen denomination of *Peronospora farinosa* f. sp. *spinaciae* in Characteristic 17

(b) Addition of "Race Pfs: 11" in Characteristic 17, including example varieties, as follows:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **17.** **(+)** | **VG** | **Resistance to *Peronospora farinosa* f. sp. *spinaciae*** | **Résistance à *Peronospora farinosa* f. sp. *spinaciae*** | **Resistenz gegen *Peronospora farinosa* f. sp. *spinaciae*** | **Resistencia a *Peronospora farinosa* f. sp. *spinaciae*** | | |
| **17.10** | | **Race Pfs: 11** | **Race Pfs: 11** | **Pathotyp Pfs: 11** | **Raza Pfs: 11** | | |
| | | absent | absente | fehlend | ausente | Lazio | 1 |
| | | present | présente | vorhanden | presente | Boeing, Califlay, Campania, Lion | 9 |

(c) Addition of a column for "Pfs: 11" in the table of differential varieties to identify races in Ad. 17, as follows:

Differential varieties to identify races:

Races Pfs: 1-8 and 10-11 of *Peronospora farinosa* f. sp. *spinaciae* are defined with a standard set of "differential varieties" according to the following table:

| **Differential variety** | Pfs: 1 | Pfs: 2 | Pfs: 3 | Pfs: 4 | Pfs: 5 | Pfs: 6 | Pfs: 7 | Pfs: 8 | Pfs: 10 | Pfs: 11 |
|---|---|---|---|---|---|---|---|---|---|---|
| Viroflay | S | S | S | S | S | S | S | S | S | S |
| Resistoflay | R | R | S | S | S | S | S | S | S | S |
| Califlay | R | S | R | S | R | S | S | R | S | R |
| Clermont | R | R | R | R | S | S | S | S | S | S |
| Campania | R | R | R | R | R | S | R | S | S | R |
| Boeing | R | R | R | R | R | R | R | S | S | R |
| Lion | R | R | R | R | R | R | R | R | S | R |
| Lazio | R | R | R | R | R | R | R | R | R | S |

Legend: R = resistance present; S = resistance absent, susceptible

PARTIAL REVISION OF THE TEST GUIDELINES FOR MANDARINS (CITRUS; GROUP 1) (DOCUMENT TG/201/1)

4. At its forty-first session held in Cuernavaca, Morelos State, Mexico, from September 27 to October 1, 2010, the Technical Working Party for Fruit Crops (TWF) discussed document TWF/41/28, as presented by Mr. Pedro Miguel Chomé Fuster and Mr. Guillermo Soler Fayos (Spain).

5. The TWF agreed to propose to the Technical Committee to adopt the partial revision of the Test Guidelines for Mandarin on the basis of document TWF/41/28 (copy provided at *http://www.upov.int/export/sites/upov/restrict/en/twf/41/twf_41_28.pdf*) with the reservation of experts from Morocco with regard to the proposed new characteristic (after characteristic 98) "Fruit: number of seeds (controlled manual cross-pollination)", for which the experts from Morocco explained that more time was needed for study of the new characteristic. The TWF agreed that the Technical Committee should be invited to consider the "Comments of Morocco concerning the new characteristics proposed 'Fruit: number of seeds (controlled manual crosspollination) and pollen viability in the UPOV Test Guidelines for Mandarin", as set out in Annex VI to this document, in conjunction with its consideration of the proposed partial revision of the Test Guidelines for Mandarin.

6. At its meeting on January 6, 2011, the Enlarged Editorial Committee (TC-EDC) concluded that there were technical issues to be resolved and recommended that those issues be referred back to TWF for further consideration.

*7. The TC is invited to consider the information in this document in conjunction with document TC/47/2.*

[Annexes follow]

ANNEX I

Amendment of characteristic 39 in the Table of Characteristics

***Proposed amendments are indicated by:***

*highlighting: proposed addition*
*~~strikethrough~~: proposed deletion*

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **39.**<br>**(+)** | **VG**<br>**QL** | **Resistance to downy mildew (*Bremia lactucae*)** | **Résistance au mildiou (*Bremia lactucae*)** | **Resistenz gegen Falschen Mehltau (*Bremia lactucae*)** | **Resistencia al mildiú (*Bremia lactucae*)** | | |
| **39.1** | **(b) (c)** | **Isolate Bl: 2** | **Isolat Bl: 2** | **Isolat Bl: 2** | **Aislado Bl: 2** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, ~~Hilde II~~, Green Towers | 1 |
| | | present | présente | vorhanden | presente | Ninja | 9 |
| **39.2** | **(c)** | **Isolate Bl: 5** | **Isolat Bl: 5** | **Isolat Bl: 5** | **Aislado Bl: 5** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, ~~Hilde II~~, Green Towers | 1 |
| | | present | présente | vorhanden | presente | Sabine | 9 |
| **39.3** | **(c)** | **Isolate Bl: 7** | **Isolat Bl: 7** | **Isolat Bl: 7** | **Aislado Bl: 7** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, ~~Hilde II~~, Green Towers | 1 |
| | | present | présente | vorhanden | presente | Valmaine, ~~Verpia~~ | 9 |
| **39.4** | **(c)** | **Isolate Bl: 12** | **Isolat Bl: 12** | **Isolat Bl: 12** | **Aislado Bl: 12** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, ~~Hilde II~~, Green Towers | 1 |
| | | present | présente | vorhanden | presente | ~~Danilla, Geisha,~~ Dandie, UCDM12 | 9 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **39.5** | **(c)** | **Isolate Bl: 14** | **Isolat Bl: 14** | **Isolat Bl: 14** | **Aislado Bl: 14** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, ~~Hilde~~, Green Towers | 1 |
| | | present | présente | vorhanden | presente | ~~Santis, Sifra, Verpia~~, Colorado, Ninja | 9 |
| **39.6** | **(c)** | **Isolate Bl: 15** | **Isolat Bl: 15** | **Isolat Bl: 15** | **Aislado Bl: 15** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, ~~Hilde II~~, Green Towers | 1 |
| | | present | présente | vorhanden | presente | ~~Mirian~~,. Colorado, Sabine | 9 |
| **39.7 (*)** | **(c)** | **Isolate Bl: 16** | **Isolat Bl: 16** | **Isolat Bl: 16** | **Aislado Bl: 16** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, Green Towers, ~~Hilde II~~ | 1 |
| | | present | présente | vorhanden | presente | Argelès, Ninja | 9 |
| **39.8** | **(c)** | **Isolate Bl: 17** | **Isolat Bl: 17** | **Isolat Bl: 17** | **Aislado Bl: 17** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, Green Towers, ~~Hilde II~~ | 1 |
| | | present | présente | vorhanden | presente | Argelès, Ninja | 9 |
| **39.9** | **(c)** | **Isolate Bl: 18** | **Isolat Bl: 18** | **Isolat Bl: 18** | **Aislado Bl: 18** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, Green Towers, ~~Hilde II~~ | 1 |
| | | present | présente | vorhanden | presente | Argelès, Ninja | 9 |
| **39.10** | **(c)** | **Isolate Bl: 20** | **Isolat Bl: 20** | **Isolat Bl: 20** | **Aislado Bl: 20** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, Green Towers, ~~Hilde II~~ | 1 |
| | | present | présente | vorhanden | presente | Argelès, Ninja | 9 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **39.11** | **(c)** | **Isolate Bl: 21** | **Isolat Bl: 21** | **Isolat Bl: 21** | **Aislado Bl: 21** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, Green Towers, ~~Hilde II~~ | 1 |
| | | present | présente | vorhanden | presente | Argelès, Colorado ~~Ninja~~ | 9 |
| **39.12** | **(c)** | **Isolate B1: 22** | **Isolat Bl: 22** | **Isolat Bl: 22** | **Aislado Bl: 22** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, Green Towers, ~~Hilde II~~ | 1 |
| | | present | présente | vorhanden | presente | Discovery, Ninja, ~~Coralis, Torpedo~~ | 9 |
| **39.13** | **(c)** | **Isolate B1: 23** | **Isolat Bl: 23** | **Isolat Bl: 23** | **Aislado Bl: 23** | | |
| | | absent | absente | fehlend | ausente | Cobham Green, Green Towers, ~~Hilde II~~ | 1 |
| | | present | présente | vorhanden | presente | Colorado, Discovery, Ninja | 9 |
| **39.14** | **(c)** | **Isolate B1: 24** | **Isolat Bl: 24** | **Isolat Bl: 24** | **Aislado Bl: 24** | | |
| | | absent | absente | fehlend | ausente | Argelès, Colorado | 1 |
| | | present | présente | vorhanden | presente | Dandie, UC DM14, PIVT 1309 | 9 |
| **39.15** | **(c)** | **Isolate B1: 25** | **Isolat Bl: 25** | **Isolat Bl: 25** | **Aislado Bl: 25** | | |
| | | absent | absente | fehlend | ausente | Colorado, ~~Penlake~~ Pennlake | 1 |
| | | present | présente | vorhanden | presente | ~~Angela~~Argelès, Ninja | 9 |
| **39.16** | **(c)** | **Isolate B1: 26** | **Isolat Bl: 26** | **Isolat Bl: 26** | **Aislado Bl: 26** | | |
| | | absent | absente | fehlend | ausente | Colorado, Discovery | 1 |
| | | present | présente | vorhanden | presente | Balesta, Bedford | 9 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **39.17** | **(c)** | **Isolate B1: 27** | **Isolat Bl: 27** | **Isolat Bl: 27** | **Aislado Bl: 27** | | |
| | | absent | absente | fehlend | ausente | Balesta, Green Towers | 1 |
| | | present | présente | vorhanden | presente | Bedford | 9 |

[Annex II follows]

ANNEX II

Amendment of Ad. 39 in Chapter 8

The current wording is reproduced on pages 1 to 6.
The proposed new wording is indicated on pages 7 to 9.

***Current wording:***

Ad. 39: Resistance to downy mildew (*Bremia lactucae*)

Useful Dm-Genes

DUS examiners should test for Dm-genes of practical value which are directly involved in giving useful resistance in lettuce varieties, and obscure or irrelevant Dm-genes need not routinely be tested.

The currently useful Dm-genes are: 2, 3, 5/8, 6, 7, 11, 14, 16 and 18, as well as R17, R36, R37 and R38 factors. Only these should be tested on a routine basis.

Special Tests

Special tests may be required for Dml, Dm4, Dm15 and Dm10 (useful in the United States of America and Australia).

If breeders claim the presence of Dm-genes other than those mentioned above, then they should state in the Technical Questionnaires how the presence of these genes could be detected and, if appropriate, submit the relevant *Bremia* isolate to the testing centre to verify the claim. Special tests may be carried out for other Dm-genes if claimed by breeders as being appropriate for DUS examination.

*Bremia* Races

The following *Bremia* races should be used to determine whether a lettuce variety possesses the Dm-genes listed above: Bl:2, Bl:5, Bl:7, Bl:12, Bl:14, Bl:15, Bl:16, Bl:17, Bl:18, Bl:20, Bl:21, Bl:22, Bl:23, Bl:24 and Bl:25. For special discrimination between Dm 5/8 and Dm 7, Bl:7 is proposed.

These isolates possess a wide range of virulences. For details, please refer to relevant literature.

New Isolates

Additional isolates could be added to test for any useful new Dm-genes that might arise.

If new isolates of *Bremia* arise that can either detect novel Dm-genes in lettuce varieties or effectively replace an isolate listed above, then these isolates should be added to those listed.

Testing of *Bremia* Isolates

There are two centres, the “Station nationale d’essais de semences” (SNES) in France and the NAK Tuinbouw in the Netherlands, which would verify and test the isolates listed above and any new isolates that are used in routine tests. These centres should make these verified isolates available, against payment of prescribed fees, to the testing centres of other UPOV members.

The addresses of the centres are as follows:

Station nationale d’essais de semences (SNES)
Rue Georges Morel
B.P. 24
49071 Beaucouzé Cedex
France
Tél. : +33 (0) 2 41 22 58 00
Tlcp. : +33 (0) 2 41 22 58 01
Mél. : service.clients@geves.fr

NAK Tuinbouw
Sotaweg 20
P.O. Box 40
2370 AA Roelofarendsveen
Pays-Bas
Tél. : + 31 (0) 71 332 62 62
Tlcp. : + 31 (0) 71 332 63 63
Mél. : info@naktuinbouw.nl

Table of *Bremia* differentials:

| | | Variety | Cobham Green | | Lednicky | UC DM2 | Dandie | R4T57D | Valmaine | Sabine | | LSE 57/15 | UC DM10 | Capitan | Hilde II | Penmlake | UC DM14 | | PIVT 1309 | LSE /18 | LS-102 | Colorado | Ninja | Discovery | | Argeles | Sextet code |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dm nr/R nr | | 0 | | 1 | 2 | 3 | 4 | 5/8 | 6 | | 7 | 10 | 11 | 12 | 13 | 14 | | 15 | 16 | 17 | 18/ | 36 | 37 | | 38 | |
| | Sextet nr | | | | 1 | 2 | 3 | 4 | 5 | 6 | | 7 | 8 | 9 | 10 | 11 | 12 | | 13 | 14 | 15 | 16 | 17 | 18 | | 19 | |
| | Sextet value | | | | 1 | 2 | 4 | 8 | 16 | 32 | | 1 | 2 | 4 | 8 | 16 | 32 | | 1 | 2 | 4 | 8 | 16 | 32 | | 1 | |
| IBEB code | Alias | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bl:1 | NL 1 | | + | | + | + | - | + | - | - | | - | + | - | + | + | + | | - | - | - | - | - | - | | - | BL-A 11/58/00/00 |
| Bl:2 | NL 2 | | + | | + | + | + | + | + | + | | - | + | (-) | + | + | + | | - | - | - | (-) | - | - | | + | BL-A 63/58/00/01 |
| Bl:3 | NL 3 | | + | | - | - | - | + | + | + | | + | + | - | + | + | (+) | | + | - | - | - | - | (-) | | - | BL-A 56/59/01/00 |
| Bl:4 | NL 4 | | + | | + | + | - | + | + | (-) | | + | + | (-) | + | + | + | | - | (-) | - | (-) | - | - | | - | BL-A 27/59/00/00 |
| Bl:5 | NL 5 | | + | | + | - | + | - | - | - | | + | + | - | + | + | - | | + | - | - | - | - | - | | - | BL-A 05/27/01/00 |
| Bl:6 | NL 6 | | + | | + | + | - | + | + | (-) | | - | + | + | + | + | + | | - | (-) | - | - | - | - | | - | BL-A 27/62/00/00 |
| Bl:7 | NL 7 | | + | | + | + | + | + | - | + | | + | + | - | + | + | + | | - | - | - | - | - | - | | - | BL-A 47/59/00/00 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bl:10 | NL 10 | | + | | + | + | + | + | + | + | | + | + | (-) | + | + | (+) | | (-) | - | - | - | - | - | | - | BL-A 63/59/00/00 |
| Bl:11 | NL 11 | | + | | + | - | - | + | + | + | | + | + | - | + | + | + | | + | + | - | - | - | - | | - | BL-A 57/59/03/00 |
| Bl:12 | NL 12 | | + | | + | - | - | + | + | + | | + | + | + | + | + | + | | + | + | - | - | - | - | | - | BL-A 57/63/03/00 |
| Bl:13 | NL 13 | | + | | + | - | + | - | + | (-) | | + | + | + | + | + | + | | - | - | - | - | - | - | | - | BL-A 21/63/00/00 |
| Bl:14 | NL 14 | | + | | + | + | + | + | + | + | | - | + | + | + | + | + | | - | - | - | - | - | - | | - | BL-A 63/62/00/00 |
| Bl:15 | NL 15 | | + | | + | + | + | + | + | - | | + | + | + | + | + | - | | - | - | - | - | - | - | | - | BL-A 31/31/00/00 |
| Bl:16 | NL 16/BL-16 | | + | | + | + | + | + | + | + | | + | + | + | + | + | - | | - | + | - | - | - | - | | - | BL-A 63/31/02/00 |
| Bl:17 | BL-17 | | + | | - | + | + | - | + | - | | + | + | - | + | + | + | | + | - | - | + | - | + | | - | BL-A 22/59/41/00 |
| Bl:18 | BL-18 | | + | | + | + | - | + | + | + | | + | + | + | + | + | - | | - | + | - | + | - | - | | - | BL-A 59/31/10/00 |
| Bl:19 | BL-19 | | + | | + | + | + | + | + | + | | - | + | + | + | + | + | | - | - | - | - | - | - | | + | BL-A 63/62/00/01 |

| | | Variety | Cobham Green | | Lednicky | UC DM2 | Dandie | R4T57D | Valmaine | Sabine | | LSE 57/15 | UC DM10 | Capitan | Hilde II | Pennlake | UC DM14 | | PIVT 1309 | LSE /18 | LS-102 | Colorado | Ninja | Discovery | | Argeles | Sextet code |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bl:20 | BL-20 | | + | | + | + | + | + | + | + | | + | + | + | + | + | - | | - | + | - | + | - | - | | - | BL-A 63/31/10/00 |
| Bl:21 | BL-21 | | + | | + | + | + | + | + | + | | + | + | + | + | + | - | | + | + | - | - | + | + | | - | BL-A 63/31/51/00 |
| Bl:22 | BL-22 | | + | | + | + | - | + | + | + | | + | + | + | + | + | + | | + | - | - | + | - | - | | - | BL-A 59/63/09/00 |
| Bl:23 | BL-23 | | + | | + | + | + | + | + | + | | + | + | + | + | + | - | | - | + | - | - | - | - | | + | BL-A 63/31/02/01 |
| Bl:24 | BL-24 | | + | | + | + | - | + | + | + | | + | + | + | + | + | - | | - | + | - | + | - | - | | + | BL-A 59/31/10/01 |
| Bl:25 | BL-25 | | + | | + | + | - | + | + | + | | + | + | + | + | + | - | | - | + | - | + | - | + | | - | BL-A 59/31/42/00 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | S1 | | + | | + | - | + | + | + | + | | + | + | - | + | + | + | | + | - | - | - | - | - | | - | BL-A 61/59/01/00 |
| | SF1 | | + | | + | + | + | - | + | - | | + | + | - | + | + | + | | + | + | - | - | - | + | | - | BL-A 23/59/35/00 |
| | IL4 | | + | | + | + | - | + | + | - | | + | + | + | + | + | + | | + | + | - | - | - | - | | + | BL-A 27/63/03/01 |
| | CS9 | | + | | + | + | + | + | + | + | | + | + | + | + | + | + | | + | - | - | - | - | - | | - | BL-A 63/63/01/00 |
| | TV | | + | | + | + | + | + | + | + | | + | + | - | + | + | + | | + | + | - | - | - | - | | - | BL-A 63/59/03/00 |

"+": susceptible
"-": resistant
"(-)": incomplete resistance
"(+)" incomplete susceptibility

Use of the sextets method to describe the resistance of varieties of lettuce to *Bremia*:

The resistance genes or Dm factors are grouped together in sixes (sextet):

1st sextet : 1, 2, 3, 4, 5/8, 6
2nd sextet : 7, 10, 11, 12, 13, 14
3rd sextet : 15, 16, 17, 18, 36, 37
4th sextet : 38

Each resistance gene or Dm factor receives a sextet number and each sextet number has a specific value (see table below).

Within each sextet, the values are allocated as follows:

- race overcoming the gene or Dm factor – (+) = sextet value
- race not overcoming the gene or Dm factor – (-) = 0 value.

All these values are then added together within the sextet in order to obtain an overall number per sextet. This number allows the race virulence spectrum to be found (only one virulence combination can correspond to a sextet value).

| | 1st sextet | | | | | | 2nd sextet | | | | | | 3rd sextet | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dm | 1 | 2 | 3 | 4 | 5/8 | 6 | 7 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | … |
| Sextet number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | … |
| Sextet value | 1 | 2 | 4 | 8 | 16 | 32 | 1 | 2 | 4 | 8 | 16 | 32 | 1 | 2 | … |
| Sextet value | + | + | - | + | - | - | - | + | - | + | + | + | - | - | |
| | 1 + 2 + 0 + 8 + 0 + 0 = 11 | | | | | | 0 + 2 + 0 + 8 + 16 + 32 = 58 | | | | | | 0 + 0 | | … |

Thus, a race with a maximum value of 63 for a sextet is virulent on all the genes or Dm and, conversely, a 0 value characterizes non-virulence on the six genes or Dm of the same sextet.

Resistance Testing Methods

The following guidelines are suggested for *Bremia* testing:

(a) Maintenance: *Bremia* races should be maintained on varieties possessing no known Dm-genes, or only obscure Dm-genes, e.g. Cobham Green, Lobjoits Green Cos, Hilde (Dm12), Olof. An alternative would be to use varieties/breeding lines which are selective for each particular isolate. The purity and quality of these maintenance varieties is important and it may be necessary to commission a seed producer to produce an adequate supply of good quality seed.

(b) Host differentials: Standard control varieties, that express the resistance genes that are being tested for, should always be used in tests, as a check. These standard varieties are available from GEVES Brion in France and NAK Tuinbouw, Netherlands:

GEVES Brion
Domaine de la Boisselière
49250 Brion
France

NAK Tuinbouw
Sotaweg 20, P.O. Box 40
2370 AA Roelofarendsveen
Netherlands

(c) Sample Size: At least 30 separate plants of each variety should be tested to establish the uniformity of the variety's Dm-gene component.

(d) Temperature: Incubation of inoculated seedlings or leaf discs should be at 15-18ºC.

(e) Inoculum Concentration: The optimum is around $1 \times 10^5$ spores per ml; at least $3 \times 10^4$ should be used. If inoculated seedlings are used, they may be inoculated prior to the emergence of the first leaf.

(f) Illumination: Adequate illumination should be provided for good plant growth. Seedlings should have fully expanded cotyledons and the plants should not be etiolated.

(g) Recording: The recording time should be as follows:

- First recording: when the control has maximum sporulation;
- Second recording: 3 days after first recording;
- Third recording: 3 days after second recording.

(In case of resistant varieties some plants may show leaf necrosis at the first recording.)

***Proposed new wording***

Ad. 39: Resistance to downy mildew (*Bremia lactucae*)

Availability of *Bremia* isolates and differentials

The "Station nationale d'essais de semences" (SNES) in France and Naktuinbouw in the Netherlands verify and test *Bremia* isolates as defined and denominated by the International *Bremia* Evaluation Board (IBEB). SNES and Naktuinbouw are responsible for delivery of denominated isolates to the testing centres of other UPOV members against payment of prescribed fees.

The addresses of the centres are as follows:

Station nationale d'essais de semences (SNES)
Rue Georges Morel
B.P. 24
49071 Beaucouzé Cedex
France
Tél. : +33 (0) 2 41 22 58 00
Tlcp. : +33 (0) 2 41 22 58 01
Mél. : service.clients@geves.fr

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

The common differential set of lettuce varieties and lines for determination of Bremia isolates is available from Naktuinbouw in the Netherlands (address as above) and GEVES in France at the following address:

GEVES Brion
Domaine de la Boisselière
49250 Brion
France

Resistance Testing Methods

(a) Maintenance: *Bremia* races may be maintained on varieties or breeding lines which are more or less selective for each particular isolate. It is essential to multiply Bl: 27 on selective plant material e.g. NunDm17.

(b) Host differentials: The host differential set that can distinguish all important *Bremia* races should always be used in tests, as a check on the identity of the isolate.

(c) Sample Size: minimum 30 plants

(d) Temperature: Incubation of inoculated seedlings or leaf discs should be at 15-18ºC.

(e) Inoculum Concentration: The optimum is around $1 \times 10^5$ spores per ml; at least $3 \times 10^4$ should be used.

(f) Illumination: Adequate illumination should be provided for good plant growth. Seedlings should have fully expanded cotyledons and the plants should not be etiolated.

(g) Recording: The recording time should be after 7, 10 and 13 days, or two of these three times. The time of maximum sporulation should occur in this period.

(h) Substrate: Seedling tests may be conducted on potting soil substrate or a substrate of paper wetted with a suitable mineral nutrient solution. Leaf disc tests may be conducted on wet paper without nutrients. Generally, the test on soil substrate will give a better discrimination of resistance and susceptibility.

(i) Observation and interpretation:

| Table legend | Observation | Interpretation |
|---|---|---|
| + | Abundant or normal sporulation on cotyledons | Susceptible |
| (+) | Normal sporulation and necrotic spots | Susceptible |
| (-) | Necrosis and (sometimes) sparse sporulation on cotyledons | Resistant |
| - | no symptoms | Resistant |

Table of *Bremia* differentials and races:

| Isolates / Differentials | Green Towers | Lednicky | UC DM2 | Dandie | R4T57D | Valmaine | Sabine | LSE 57/15 | UC DM10 | Capitan | Hilde II | Pennlake | UC DM14 | NunDm15 | LSE/18 | NunDm17 | Colorado | Ninja | Discovery | Argelès | RYZ 2164 | RYZ 910457 | Bedford | Balesta | Bellissimo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bl: 1 | + | + | + | - | + | - | - | - | + | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| Bl: 2 | + | + | + | + | + | + | + | - | + | (-) | + | + | + | - | - | - | (-) | - | - | + | - | - | - | + | + |
| Bl: 3 | + | - | - | - | + | + | + | + | + | - | + | + | (+) | + | - | - | - | - | (-) | - | - | - | - | - | + |
| Bl: 4 | + | + | + | - | + | + | (-) | + | + | (-) | + | + | + | - | (-) | - | (-) | - | - | - | - | - | - | - | - |
| Bl: 5 | + | + | - | + | - | - | - | + | + | - | + | + | - | + | - | - | - | - | - | - | - | (-) | - | - | - |
| Bl: 6 | + | + | + | - | + | + | (-) | - | + | + | + | + | + | - | - | - | - | - | - | - | - | (-) | - | - | - |
| Bl: 7 | + | + | + | + | + | - | + | + | + | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| Bl: 10 | + | + | + | + | + | + | + | + | + | (-) | + | + | (+) | - | - | - | - | - | - | - | - | (-) | - | - | - |
| Bl: 11 | + | + | - | - | + | + | + | + | + | - | + | + | + | + | + | - | - | - | - | - | | | | | - |
| Bl: 12 | + | + | - | - | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - |
| Bl: 13 | + | + | - | + | - | + | (-) | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| Bl: 14 | + | + | + | + | + | + | + | - | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| Bl: 15 | + | + | + | + | + | + | - | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Bl: 16 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | - | - | - | - | - | - | - | - | - | - |
| Bl: 17 | + | - | + | + | - | + | - | + | + | - | + | + | + | + | - | - | + | - | + | - | - | - | (+) | - | - |
| Bl: 18 | + | + | + | - | + | + | + | + | + | + | + | + | - | - | + | - | + | - | - | - | - | - | - | - | - |
| Bl: 20 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | - | + | - | - | - | - | - | - | - | - |
| Bl: 21 | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | - | - | + | + | - | - | - | (-) | - | - |
| Bl: 22 | + | + | + | - | + | + | + | + | + | + | + | + | + | + | - | - | + | - | - | - | - | - | (-) | + | - |
| Bl: 23 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | - | - | - | - | + | - | - | - | - | - |
| Bl: 24 | + | + | + | - | + | + | + | + | + | + | + | + | - | - | + | - | + | - | - | + | - | - | - | - | - |
| Bl: 25 | + | + | + | - | + | + | + | + | + | + | + | + | - | - | + | - | + | - | + | - | - | - | - | - | - |
| Bl: 26 | + | + | + | + | + | + | (+) | + | + | + | + | + | - | - | + | - | + | + | + | + | - | - | - | - | - |
| Bl: 27 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | - | - | + | + | - | - | + | - |

Note on table of Bremia differentials

The differential NunDm17 is a replacement for Ls102. The differential NunDm15 is a replacement for PIVT 1309. Ls102 and PIVT1309 have the same resistance pattern but poor germinability. More detailed information about the use of the table can be found in the relevant literature in chapter 9.

[Annex III follows]

ANNEX III

Addition of characteristic 41 “Resistance to *Nasonovia ribisnigri* biotype Nr: 0”

***Proposed wording:***

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **41**<br><br>**(+)** | **VG** | **Resistance to *Nasonovia ribisnigri* biotype Nr: 0** | **Résistance au *Nasonovia ribisnigri* biotype Nr: 0** | **Resistenz gegen *Nasonovia ribisnigri* Biotyp Nr: 0** | **Resistencia al *Nasonovia ribisnigri* biotype Nr: 0** | | |
| | | absent | absente | fehlend | ausente | Green Towers, Abel, Nadine | 1 |
| | | present | présente | vorhanden | presente | Silvinas, Barcelona, Dynamite | 9 |

[Annex IV follows]

ANNEX IV

Addition of an explanation in Chapter 8 Ad. 41

***Proposed wording:***

Ad. 41: Resistance to *Nasonovia ribisnigri* biotype Nr: 0

Maintenance of biotype

*Nasonovia ribisnigri* is a leaf aphid and may be maintained alive on susceptible lettuce plants in aphid-proof chambers or tents in a glasshouse. *N. ribisnigri* is usually green, but some biotypes are red. A red aphid is easier to see on a green leaf. Therefore red biotypes are usually preferable. The aphid's body size is 1.5-2.5 mm. The body has 7 dark spots. The ends of the legs are black.

The common biotype Nr: 0 can be distinguished from resistance-breaking biotypes by means of a biotest using a suitable resistant control variety, for example Silvinas.

Multiplication:
On a susceptible variety at 20-22°C for 10-14 days. Aphids are shaken off into a Petri-dish.

Sowing:
12°C for germination and early growth; plant distance at least 5 cm.
Number of plants to be tested: 28.

Inoculation method:
Careful transfer of 5 aphids per plant using a fine paintbrush.
Plant stage at inoculation: 15 days.
Temperature: 20-22°C.

Observation:
First observation: 10 days after inoculation.
Second observation: daily check whether newborn aphids are mature (= red).
End of test: max. 15 days after inoculation.

Observation at end of test: Count the number of mature (= red) aphids on each plant.

| Scale for observations: | | Interpretation of data |
|---|---|---|
| 0 | no aphids | Resistant |
| 1 | 1-5 aphids per plant | Resistant |
| 2 | 6-10 aphids per plant | Undecided |
| 3 | > 10 aphids per plant | Susceptible |

Remarks
Resistant control varieties and susceptible control varieties should have at least 95% (26/28) resistant plants and susceptible plants, respectively.
If more than 2 of 28 plants of the control varieties are undecided or off type, the experiment should be repeated.

[Annex V follows]

ANNEX V

Addition of item to Chapter 9

*The following literature should be added to Chapter 9:*

"Van der Arend et al., 2007: Identification and nomination of new races of *Bremia lactucae* in Europe by IBEB until 2006. Eucarpia Leafy Vegetables 2007 Conference Abstracts, 18-20 April 2007, University of Warwick, Poster presentations, pp. 27 v.v."

[Annex VI follows]

ANNEX VI

# COMMENTS OF MOROCCO CONCERNING THE NEW CHARACTERISTICS PROPOSED "FRUIT: NUMBER OF SEEDS (CONTROLLED MANUAL CROSSPOLLINATION) AND POLLEN VIABILITY IN THE UPOV TEST GUIDELINE FOR MANDARIN

**Comment 1**: **Ovule fertility**

In the UPOV test guideline to test the ovule fertility we can find only the character nº 99 "Fruit: number of seeds (open pollination)". This character corresponds to the study of female fertility under open-pollination conditions.
The Spanish proposal on this character, namely the female fertility involves the introduction of a new character 98 bis, which is the study of female fertility by cross-pollination.

**However:**

**1**- It was shown that the study of female fertility in citrus is very effective under open pollination conditions than hand pollination (**Masahi et al., 1995**).

**2- Brown and Krezdorn**. **(1969)**, reported that standard pollination tests involving massive applications of pollen alone are not sufficient to delineate those varieties which are good pollinators and to distinguish the degree of female fertility.
Indeed, They do not take into account species or variety preference by the bees, the amount of pollen carried by bees, the number of visits bees make to citrus flowers and the amount of pollen produced by flowers of given varieties. These factors ignored in the new proposed character are taken into account in the character 99 (UPOV test guideline) which corresponds to the study of female fertility.

**Comment 2: Pollen viability**

In the original version (Ch4.2: Choice of characters, UPOV Guidelines for the mandarin), the character 25 "Anthers: pollen viable," is noted by two states of expression: "absent or present. To change this character by the addition of different expression levels of pollen viability, the Spanish proposal was based on the fact that the number of seed in the fruit depends on the pollen viability.

**However:**

**1**-In a study it was reported that The reduced seediness in the Orlando tangelos set by Minneola pollen cannot be attributed to low viability of the pollen because Minneola pollen on King orange flowers produced the highest degree e of seediness of all the combinations tested, with an average of 30 seeds in King fruit (**Philip. et al. 1961**).

**2- Masashi et al. (2006)** in a study designed to investigate the compatibility and incompatibility between the tangerine and the variety Ariake it was shown that pollen tube growth in styles of Ariake x clementine and reciprocal cross combination, Clementine x Ariake was inhibited, although both accessions could produces viable pollen.

The number of seed in the fruits depends on compatibility of the pollen with the stigma of the female variety, and pollen viability rather than only the degree of pollen viability.

**Conclusions**

Based on these arguments, the ovule fertility can be estimated by open pollination rather than hand pollination and the new character proposed by *expert from Spain* would not be added in the UPOV
The number of seed in the fruits depends on pollen compatibility with the stigma of the female variety, and pollen viability rather than only the degree of pollen viability.

On the other hand we support the remarks made by Australian delegation concerning the conditions of experimentation regarding hand pollination. This supposes that in experimentation we should use a source of pollen which in practice is not practicable.

**References:**

**Brown H. D. and Krezdorn A. H. 1969**. Hand and pollination tests and field evaluation of pollinators for citrus . FLORIDA STATE HORTICULTURAL SOCIETY.,

**Philip C. Reece. Robert O. Register. 1961**. Influence of pollinators on fruit set on robinson and Osceola tangerine hybrids. 1961. Florida state horticultural society.

**Massashi Y., Tatsuya K., Shigeto T. 2006**. Self-and cross-incompatibility of various Citrus Accesions. J . japan. Soc. Hort. 75 (5), 372-378.

**Massashi Y., Ryoji M., and Yoshio Y., 1995.** Relationship between sterility and seedlessness in citrus. Japan. Soc. Hort. Sci 64 (1): 23-29.

[End of Annexes and of document]