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**WORKING PAPER ON *BREMIA LACTUCAE*
PROPOSED PARTIAL REVISION OF TEST GUIDELINES FOR LETTUCE
(DOCUMENT TG/13/10)**

Document prepared by an expert from France

1. The following changes are proposed as a basis for a partial revision of the Test Guidelines for Lettuce (document TG/13/10):
 - (a) inclusion of “Isolate Bl 26” in Characteristic 39 of the Table of Characteristics and corresponding modifications in the table of *Bremia* differentials under Ad. 39 in Chapter 8.
 - (b) inclusion of a new sub-section (h) Recording Method and Interpretation of the Data (Based on a Test in Cotyledon Stage) under the section “Resistance Testing Methods” in Ad. 39 in Chapter 8.
2. Annex I and Annex II to this document reproduce, respectively, Characteristic 39 and Ad. 39, with the proposed changes.
3. The changes above would result in the partial revision of the Test Guidelines for Lettuce (document TG/13/10 Rev.).

[Annex I follows]

ANNEX I

39.	VG	Resistance to downy mildew (<i>Bremia lactucae</i>)	Résistance au mildiou (<i>Bremia lactucae</i>)	Resistenz gegen Falschen Mehltau (<i>Bremia lactucae</i>)	Resistencia al mildiú (<i>Bremia lactucae</i>)		
	(+)						
QL							
39.1	(b) (c)	Isolate Bl:2	Isolat Bl:2	Isolat Bl:2	Aislado Bl:2		
		absent	absente	fehlend	ausente	Hilde II	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	Hilde II	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	Hilde II	1
		present	présente	vorhanden	presente	Verpia	9
39.4	(c)	Isolate Bl:12	Isolat Bl:12	Isolat Bl:12	Aislado Bl:12		
		absent	absente	fehlend	ausente	Hilde II	1
		present	présente	vorhanden	presente	Danilla, Geisha	9
39.5	(c)	Isolate Bl:14	Isolat Bl:14	Isolat Bl:14	Aislado Bl:14		
		absent	absente	fehlend	ausente	Hilde	1
		present	présente	vorhanden	Presente	Santis, Sifra, Verpia	9
39.6	(c)	Isolate Bl:15	Isolat Bl:15	Isolat Bl:15	Aislado Bl:15		
		absent	absente	fehlend	ausente	Hilde II	1
		present	présente	vorhanden	presente	Mirian	9
39.7	(c) (*)	Isolate Bl:16	Isolat Bl:16	Isolat Bl:16	Aislado Bl:16		
		absent	absente	fehlend	ausente	Cobham Green, Hilde II	1
		present	présente	vorhanden	presente	Argelès, Ninja	9

39.8	(c)	Isolate BI:17	Isolat BI:17	Isolat BI:17	Aislado BI:17		
		absent	absente	fehlend	ausente	Cobham Green, Hilde II	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.9	(c)	Isolate BI:18	Isolat BI:18	Isolat BI:18	Aislado BI:18		
		absent	absente	fehlend	ausente	Cobham Green, Hilde II	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.10	(c)	Isolate BI:20	Isolat BI:20	Isolat BI:20	Aislado BI:20		
		absent	absente	fehlend	ausente	Cobham Green, Hilde II	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.11	(c)	Isolate BI:21	Isolat BI:21	Isolat BI:21	Aislado BI:21		
		absent	absente	fehlend	ausente	Cobham Green, Hilde II	1
		present	présente	vorhanden	presente	Colorado, Ninja	9
39.12	(c)	Isolate BI:22	Isolat BI:22	Isolat BI:22	Aislado BI:22		
		absent	absente	fehlend	ausente	Cobham Green, Hilde II	1
		present	présente	vorhanden	presente	Coralis, Torpedo	9
39.13	(c)	Isolate BI:23	Isolat BI:23	Isolat BI:23	Aislado BI:23		
		absent	absente	fehlend	ausente	Cobham Green, Hilde II	1
		present	présente	vorhanden	presente	Colorado	9
39.14	(c)	Isolate BI:24	Isolat BI:24	Isolat BI:24	Aislado BI:24		
		absent	absente	fehlend	ausente	Argeles, Colorado	1
		present	présente	vorhanden	presente	Dandie, UC DM14, PIVT 1309	9
39.15	(c)	Isolate BI:25	Isolat BI:25	Isolat BI:25	Aislado BI:25		
		absent	absente	fehlend	ausente	Colorado, Penlake	1
		present	présente	vorhanden	presente	Angela, Ninja	9
39.16	(c)	Isolate BI:26	Isolat BI:26	Isolat BI:26	Aislado BI:26		
		absent	absente	fehlend	ausente	Colorado, Discovery	1
		present	présente	vorhanden	presente	Arran, Bakura	9

[Annex II follows]

ANNEX II

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 and Bl:26. 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:

Isolate	Variety	Genotype		Value	Race																			
		0	1		Sextet code																			
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
BI:2	Cobham Green	+	+		+	+	+	+	+	+	-	+	(-)	+	+	+	-	-	-	-	(-)	-	-	+
BI:5	Lednický	+	+		+	-	+	-	-	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-
BI:7	UC DM2	+	+		+	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-
BI:12	Dandie	+	+		-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
BI:14	R4T57D	+	+		+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-
BI:15	Valmaine	+	+		+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-
BI:16	Sabine	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-
BI:17	LSE 57/15	+	-		+	+	+	-	+	-	+	+	-	+	+	+	+	-	-	+	-	+	-	-
BI:18	UC DM10	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-
BI:20	Capitan	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-
BI:21	Hilde II	+	+		+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-
BI:22	Pennlake	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-
BI:23	UC DM14	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+
BI:24	PIVT 1309	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	+
BI:25	LSE /18/ CG Dm 16	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-
BI:26	LS-102	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+
	Colorado	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-
	Ninja	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-
	Discovery	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-
	Argelles	+	+		+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-

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

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).

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

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 15-18°C.

(e) Inoculum Concentration: The optimum is around 1×10^5 spores per ml; at least 3×10^4 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.)

(h) Recording Method and Interpretation of the Data (based on a test in cotyledon stage) The following symptoms may be recorded:

1. Abundant white sporulation on both sides of the cotyledon
2. Normal sporulation only on the lower side of the cotyledons
3. Normal sporulation only on the lower side of the cotyledons combined with necrotic spots
4. Sparse sporulation on the lower side of the cotyledons combined with necrosis (sporebearers sprout from small necrotic spots)
5. Necrotic pinpoints
6. No symptoms

At the third recording, the interpretation of the results is as follows:

Classes 1, 2 and 3: susceptible

Classes 4, 5 and 6: resistant

Class 3: relates to incomplete susceptibility in the above mentioned table of Bremia differentials (+).

Class 4: relates to incomplete resistance in the above mentioned table of Bremia differentials (-).

[End of Annex II and of document]