



TWV/46/35

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL WORKING PARTY FOR VEGETABLES**Forty-Sixth Session
near the city of Venlo, Netherlands, June 11 to 15, 2012****PROPOSED PARTIAL REVISION OF THE TEST GUIDELINES FOR LETTUCE
(DOCUMENT TG/13/10 REV.)***Document prepared by experts from the Netherlands*

1. It is proposed to make a partial revision of the Test Guidelines for Lettuce (document TG/13/10 Rev.) in order to amend Characteristic 39. Resistance to downy mildew (*Bremia lactuca*) as follows:
 - (a) to revise the example varieties
 - (b) to provide a revised explanation for resistance to downy mildew according to the explanations for disease resistance characteristics in Test Guidelines, as set out in document TGP/12/2 Draft 2 "Guidance on Certain Physiological Characteristics", Section 2.4.
2. The Annex to this document contains the proposals as set out in paragraph 1 of this document.

[Annex follows]

ANNEX

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

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota	
39.	VG	Resistance to downy mildew (<i>Bremia lactuca</i>)	Résistance au mildiou (<i>Bremia lactuca</i>)	Resistenz gegen Falschen Mehltau (<i>Bremia lactuca</i>)	Resistencia al mildiú (<i>Bremia lactuca</i>)		
	(+)						
	QL						
39.1	(b) (c)	Isolate BI:2	Isolat BI:2	Isolat BI:2	Aislado BI:2		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers	1
		present	présente	vorhanden	presente	Ninja	9
39.2	(c)	Isolate BI:5	Isolat BI:5	Isolat BI:5	Aislado BI:5		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers	1
		present	présente	vorhanden	presente	Sabine	9
39.3	(c)	Isolate BI:7	Isolat BI:7	Isolat BI:7	Aislado BI:7		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers	1
		present	présente	vorhanden	presente	Valmaine	9
39.4	(c)	Isolate BI:12	Isolat BI:12	Isolat BI:12	Aislado BI:12		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers	1
		present	présente	vorhanden	presente	Dandie, UCDm2	9
39.5	(c)	Isolate BI:14	Isolat BI:14	Isolat BI:14	Aislado BI:14		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers	1
		present	présente	vorhanden	Presente	Colorado, Ninja	9
39.6	(c)	Isolate BI:15	Isolat BI:15	Isolat BI:15	Aislado BI:15		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers,	1
		present	présente	vorhanden	presente	Colorado, Sabine	9
39.7	(c) (*)	Isolate BI:16	Isolat BI:16	Isolat BI:16	Aislado BI:16		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers	1
		present	présente	vorhanden	presente	Argelès, Ninja	9

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
39.8	(c) Isolate BI:17	Isolat BI:17	Isolat BI:17	Aislado BI:17		
	absent	absente	fehlend	ausente	Cobham Green, Green Towers	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, Green Towers	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, Green Towers	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, Green Towers	1
	present	présente	vorhanden	presente	Argelès Colorado, Ninja	9
39.12	(c) Isolate BI:22	Isolat BI:22	Isolat BI:22	Aislado BI:22		
	absent	absente	fehlend	ausente	Cobham Green, Green Towers	1
	present	présente	vorhanden	presente	Discovery, Ninja	9
39.13	(c) Isolate BI:23	Isolat BI:23	Isolat BI:23	Aislado BI:23		
	absent	absente	fehlend	ausente	Cobham Green, Green Towers	1
	present	présente	vorhanden	presente	Colorado, Discovery, Ninja	9
39.14	(c) Isolate BI:24	Isolat BI:24	Isolat BI:24	Aislado BI:24		
	absent	absente	fehlend	ausente	Argelès, Colorado	1
	present	présente	vorhanden	presente	Dandie, UC DM14, PIVT 1309 NunDm15	9
39.15	(c) Isolate BI:25	Isolat BI:25	Isolat BI:25	Aislado BI:25		
	absent	absente	fehlend	ausente	Colorado, Discovery, Pennlake	1
	present	présente	vorhanden	presente	Argelès, Ninja	9
39.16	(c) Isolate BI:26	Isolat BI:26	Isolat BI:26	Aislado BI:26		
	absent	absente	fehlend	ausente	Colorado, Discovery	<u>1</u>
	present	présente	vorhanden	presente	Balesta, Bedford	<u>9</u>

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
39.17 (c) Isolate BI:27	Isolat BI:27	Isolat BI:27	Aislado BI:27		
absent	absente	fehlend	ausente	Balesta, <u>Colorado</u> , <u>Green Towers</u>	<u>1</u>
present	présente	vorhanden	presente	<u>Balesta</u> , Bedford, <u>Discovery</u>	<u>9</u>

Ad 39. Resistance to *Bremia lactucae* (BI)

* 1. Pathogen	<i>Bremia lactucae</i>
2. Quarantine status	-
* 3. Host species	<i>Lactuca sativa</i> L.
* 4. Source of inoculum	GEVES (France) or Naktuinbouw (The Netherlands)
* 5. Isolate	BI :1 – BI :27 (see table below)
6. Establishment isolate identity	Test on differentials
7. Establishment pathogenicity	Test on susceptible varieties
8. Multiplication inoculum	
8.1 Multiplication medium	Lettuce leaf
8.2 Multiplication variety	Susceptible variety, for example Green Towers. For higher races, 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	Normally 60, minimum 20
* 9.2 Number of replicates	-
* 9.3 Control varieties	(Informative) differentials
* 9.4 Test design	Include control varieties
9.5 Test facility	Climate room
9.6 Temperature	15°C-17°C
9.7 Light	Adequate for good plant growth; seedlings should not etiolate. 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, or on potting soil. 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 $3 \cdot 10^4$ - $1 \cdot 10^5$
*10.3 Plant stage at inoculation	Cotyledon stage
*10.4 Inoculation method	Spraying till run-off Reduced light 24 hours after inoculation
10.5 First observation	7 days after inoculation
10.6 Second observation	10 days after inoculation
*10.7 Final observations	13 days after inoculation; two of these three time points may be sufficient. 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	+ 1. Abundant sporulation on both sides of the cotyledon (+) 2. Normal sporulation on the lower side of the cotyledon (+) 3. Normal sporulation on the lower side of the cotyledons combined with necrotic spots

- (-) 4. Sparse sporulation on the lower side of the cotyledons combined with necrosis
- (-) 5. Necrotic pinpoints
- 6. No symptoms

*11.3 Validation of test
11.4 Off-types

on standards
Susceptible plants in a resistant variety
Resistant plants in a susceptible variety
3 or less off-types in 60 plants
Class 1, 2 and 3: susceptible
Class 4, 5 and 6: resistant

*12. Interpretation of data

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.

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 www.worldseed.org. The table for Bl:1-27 is given below. Some differentials and race names are printed in gray. These varieties and races are considered less important.

Isolates	Differentials	Green Towers	Lednický	UC DM2	Dandle	R4T57D	Valmaine	Sabine	LSE 57/15	UC DM10	Capitan	Hilde II	Pernlake	UC DM14	NunDm15	CGDm16	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		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	-	+	+