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UPOV

TC/49/38
ORIGINAL: English
DATE: February 7, 2013

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL COMMITTEE

Forty-Ninth Session
Geneva, March 18 to 20, 2013

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

Document prepared by the Office of the Union

1. The Technical Working Party for Vegetables (TWV) at its forty-sixth session, held near the city of Venlo, Netherlands, from June 11 to 15, 2012, agreed to propose to revise the Test Guidelines for Lettuce (document TG/13/10 Rev.) as follows:

(a) to revise the example varieties for Characteristic 39 “Resistance to downy mildew (*Bremia lactuca*)”
(b) to add a new characteristic (Characteristic 42) “Resistance to *Fusarium oxysporum* f.sp. *lactucae* (Fol), Race1” after Characteristic 41
(c) to provide a revised explanation for Characteristics 39 and an explanation for Characteristic 42 according to the explanations for disease resistance characteristics in Test Guidelines, as set out in document TGP/12/2 “Guidance on Certain Physiological Characteristics”, Section 2.4.

2. The Annex to this document contains the proposed partial revision of the Test Guidelines for Lettuce (document TG/13/10 Rev.), as set out in paragraph 1 of this document.

3. The partial revision to document TG/13/10 Rev. would be adopted as document TG/13/10 Rev.2.

[Annex follows]

ANNEX

Proposal to amend example varieties for characteristic 39 “Resistance to downy mildew (*Bremia lactucae*)”

Proposed amendments are indicated by:

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

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
39. **(+)**	**VG**	**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**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Ninja	9
39.2	**(b) (c)**	**Isolate Bl: 5**	**Isolat Bl: 5**	**Isolat Bl: 5**	**Aislado Bl: 5**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Sabine	9
39.3	**(b) (c)**	**Isolate Bl: 7**	**Isolat Bl: 7**	**Isolat Bl: 7**	**Aislado Bl: 7**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Valmaine	9
39.4	**(b) (c)**	**Isolate Bl: 12**	**Isolat Bl: 12**	**Isolat Bl: 12**	**Aislado Bl: 12**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Dandie, UCDm2	9
39.5	**(b) (c)**	**Isolate Bl: 14**	**Isolat Bl: 14**	**Isolat Bl: 14**	**Aislado Bl: 14**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	Presente	Colorado, Ninja	9
39.6	**(b) (c)**	**Isolate Bl: 15**	**Isolat Bl: 15**	**Isolat Bl: 15**	**Aislado Bl: 15**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers,	1
		present	présente	vorhanden	presente	Colorado, Sabine	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
39.7 (*)	**(b) (c)**	**Isolate Bl: 16**	**Isolat Bl: 16**	**Isolat Bl: 16**	**Aislado Bl: 16**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.8	**(b) (c)**	**Isolate Bl: 17**	**Isolat Bl: 17**	**Isolat Bl: 17**	**Aislado Bl: 17**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.9	**(b) (c)**	**Isolate Bl: 18**	**Isolat Bl: 18**	**Isolat Bl: 18**	**Aislado Bl: 18**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.10	**(b) (c)**	**Isolate Bl: 20**	**Isolat Bl: 20**	**Isolat Bl: 20**	**Aislado Bl: 20**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.11	**(b) (c)**	**Isolate Bl: 21**	**Isolat Bl: 21**	**Isolat Bl: 21**	**Aislado Bl: 21**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Argelès, Colorado, ~~Ninja~~	9
39.12	**(b) (c)**	**Isolate Bl: 22**	**Isolat Bl: 22**	**Isolat Bl: 22**	**Aislado Bl: 22**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Discovery, Ninja	9
39.13	**(b) (c)**	**Isolate Bl: 23**	**Isolat Bl: 23**	**Isolat Bl: 23**	**Aislado Bl: 23**		
QL		absent	absente	fehlend	ausente	~~Cobham Green~~, Green Towers	1
		present	présente	vorhanden	presente	Colorado, Discovery, Ninja	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
39.14	**(b) (c)**	**Isolate Bl: 24**	**Isolat Bl: 24**	**Isolat Bl: 24**	**Aislado Bl: 24**		
QL		absent	absente	fehlend	ausente	Argelès, Colorado	1
		present	présente	vorhanden	presente	Dandie, NunDm15, ~~PIVT 1309~~, UC DM14	9
39.15	**(b) (c)**	**Isolate Bl: 25**	**Isolat Bl: 25**	**Isolat Bl: 25**	**Aislado Bl: 25**		
QL		absent	absente	fehlend	ausente	Colorado, Discovery, ~~Pennlake~~	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.16	**(b) (c)**	**Isolate Bl: 26**	**Isolat Bl: 26**	**Isolat Bl: 26**	**Aislado Bl: 26**		
QL		absent	absente	fehlend	ausente	Colorado, Discovery	1
		present	présente	vorhanden	presente	Balesta, Bedford	9
39.17	**(b) (c)**	**Isolate Bl: 27**	**Isolat Bl: 27**	**Isolat Bl: 27**	**Aislado Bl: 27**		
QL		absent	absente	fehlend	ausente	Balesta, Colorado, ~~Green Towers~~	1
		present	présente	vorhanden	presente	Bedford, Discovery	9

Proposal to add new characteristic “Resistance to Fusarium oxysporum f.sp. lactucae” after characteristic 41

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
NEW **(+)**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *lactucae*** -Race 1	**Résistance à *Fusarium oxysporum* f. sp. *lactucae*** -Race 1	**Resistenz gegen *Fusarium oxysporum* f. sp. *lactucae*** -Pathotyp 1	**Resistencia a *Fusarium oxysporum* f. sp. *lactucae*** -Raza 1		
QL		susceptible	sensible	anfällig	susceptible	Cobham Green, Patriot Salinas	1
		resistant	resistante	resistent	resistente	Costa Rica No.4, Romasol	9

Proposal to amend addendum 39 “Resistance to downy mildew (*Bremia lactucae*)”

Current wording:

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

Availability of *Bremia* isolates and differentials

The GEVES in France and Naktuinbouw in the Netherlands verify and test *Bremia* isolates as defined and denominated by the International *Bremia* Evaluation Board (IBEB). GEVES 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:

GEVES
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 1×10^5 spores per ml; at least 3×10^4 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.

Proposed new wording:

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

* 1. Pathogen	*Bremia lactucae*
2. Quarantine status	-
* 3. Host species	*Lactuca sativa* L.
* 4. Source of inoculum	GEVES (France) or Naktuinbouw (The Netherlands)
* 5. Isolate	Bl :1 – Bl :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 3.10^4-1.10^5
*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 — on standards

11.4 Off-types — Susceptible plants in a resistant variety
Resistant plants in a susceptible variety
3 or less off-types in 60 plants

*12. Interpretation of data — Class 1, 2 and 3: susceptible
Class 4, 5 and 6: resistant

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	Lednicky	UC DM2	Dandie	R4T57D	Valmaine	Sabine	LSE 57/15	UC DM10	Capitan	Hilde II	Pennlake	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		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	+	-

Availability of *Bremia* isolates and differentials

The GEVES in France and Naktuinbouw in the Netherlands verify and test *Bremia* isolates as defined and denominated by the International *Bremia* Evaluation Board (IBEB). GEVES 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:

GEVES
25 Rue Georges Morel
CS 90024
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

Proposal to add addendum for new characteristic "Resistance to *Fusarium oxysporum* f.sp*. lactucae*" after characteristic 41

Ad. NEW: Resistance to *Fusarium oxysporum* f.sp. *lactucae*

-Race 1

* 1. Pathogen	*Fusarium oxysporum* f.sp*. lactucae*
2. Quarantine status	EPPO alert list
* 3. Host species	*Lactuca sativa* L.
* 4. Source of inoculum	NIAS Genebank, INRAN, Naktuinbouw, GEVES
* 5. Isolate	Fol : 1 and Fol : 2
6. Establishment isolate identity	Use microscope and inoculation to lettuce susceptible standard
7. Establishment pathogenicity	Use lettuce susceptible standard
8. Multiplication inoculum	
8.1 Multiplication medium	Inoculation by sowing on contaminated soil: Wheat bran-soil medium. Inoculation by soaking seedlings: on synthetic liquid medium (e.g. Potatoes Dextrose Broth)
8.2 Multiplication variety	
8.3 Plant stage at inoculation	See 10.3
8.4 Inoculation medium	
8.5 Inoculation method	See 10.4
8.6 Harvest of inoculum	Inoculation by sowing on contaminated soil: 7-10 day-old culture Inoculation by soaking seedlings: 15 days
8.7 Check of harvested inoculum	
8.8 Shelf life/viability inoculum	
9. Format of the test	
* 9.1 Number of plants per genotype	20 plants
* 9.2 Number of replicates	
* 9.3 Control varieties	
Susceptible	Cobham Green, Salinas, Patriot Cobham Green is slightly less and Salinas is less susceptible than Patriot
Resistant to Fol:1	Costa Rica No.4, Romasol
* 9.4 Test design	Include control varieties
9.5 Test facility	Greenhouse or climate room
9.6 Temperature	20-28 ^{o}C
9.7 Light	Under natural day length
9.8 Season	
9.9 Special measures	
10. Inoculation	
10.1 Preparation inoculum	Inoculation by sowing on contaminated soil: Wheat bran-soil medium culture are mixed with sterilized soil Inoculation by soaking seedlings: soaking of roots and of hypocotyls axis for 5 to 15 min in the inoculums suspension and transplantation of inoculated plantlets in soil
10.2 Quantification inoculum	Inoculation by sowing on contaminated soil: soil: culture =20: 1 Inoculation by soaking seedlings: spores are harvested and adjusted to 10^7 sp/mL
*10.3 Plant stage at inoculation	Inoculation by sowing on contaminated soil: seeds stimulated to emerge Remark: Avoid seeds rotted by factors other than pathogen. Inoculation by soaking seedlings: cotyledons

*10.4 Inoculation method	Two methods can be use for inoculation : by sowing seeds to contaminated soil or by soaking seedlings
10.5 First observation	After 7- 10 days from inoculation
10.6 Second observation	14 days from inoculation
*10.7 Final observations	20-25 days after inoculation (sowing or soaking)
11. Observations	
*11.1 Method	Visual and/or counting number of plants with symptom
*11.2 Observation scale	Inoculation by sowing on contaminated soil: Symptoms: stunting, wilting, dead plant As reference calculate of Disease Severity Index (DSI) and Disease Incidence(DI) 0: healthy 1: slightly stunting, growing reduction 2: severely stunting 3: die DSI = (0A + 1B + 2C + 3D) / (A + B + C + D) *A ~ D: number of plants of each category DI = (0A + 1B + 2C + 3D) *100/ ((A + B + C + D)*3) Inoculation by soaking seedlings: Symptoms: growth reduction and brown vessels above cotyledons, dead plant
*11.3 Validation of test	Analysis of results should be calibrated with results of controls
*12. Interpretation of data	Inoculation by sowing on contaminated soil: Susceptible: severely stunting, wilting, dead plant (DSI :Relative evaluation to DSI of example variety) (Race1:DI Value is higher than 10%) Resistant: no stunting, no wilting (DSI :Relative evaluation to DSI of example variety), (Race1:DI Value is lower than 10%) Inoculation by soaking seedlings: susceptible: growth reduction and brown vessels above cotyledons, dead plant Resistant: no growth reduction and no brown vessels above cotyledons

13. Critical control points:

Availability of *Fusarium oxysporum* f.sp. *lactucae* Race 1

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[End of Annex and of document]