

Technical Committee

TC/59/18

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PARTIAL REVISION OF THE TEST GUIDELINES FOR LETTUCE

Document prepared by an expert from the Netherlands

Disclaimer: this document does not represent UPOV policies or guidance

1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Lettuce (document TG/13/11 Rev. 2).

2. The Technical Working Party for Vegetables (TWV), at its fifty-seventh session¹, considered a proposal for a partial revision of the Test Guidelines for Lettuce (*Lactuca sativa* L.) on the basis of documents TG/13/11 Rev. 2 and TWV/57/12 “Partial revision of the Test Guidelines for Lettuce” and proposed the following changes (see document TWV/57/26 “Report”, paragraph 68):

- (a) Addition of “Resistance to *Bremia lactucae* (BI) Isolate BI: 36EU”, including example varieties;
- (b) Revision of explanation Ad. 38 to 52: Resistance to *Bremia lactucae* (BI).
- (c) Inclusion of characteristics from the Table of Characteristics in the Technical Questionnaire

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

Proposed addition of “Resistance to *Bremia lactucae* (BI) Isolate BI: 36EU”

Proposed addition

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
53	QL VG	(+)				
	Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 36EU	Résistance à <i>Bremia lactucae</i> (BI) Isolat BI: 36EU	Resistenz gegen <i>Bremia lactucae</i> (BI) Isolat BI: 36EU	Resistencia a <i>Bremia lactucae</i> (BI) Aislado BI: 36EU		
	absent	absente	fehlend	ausente	<u>Bartoli, RYZ2164</u>	<u>1</u>
	<u>present</u>	<u>présente</u>	<u>vorhanden</u>	<u>presente</u>	<u>Design, Kibrille</u>	<u>9</u>

4. It is proposed to include the new characteristic 53 in Chapter 10 “Technical questionnaire”, Section 5 “Characteristics of the variety to be indicated”.

¹ held in Antalya, Türkiye, from May 1 to 5, 2023

Proposed revision of explanation Ad. 38 to 52 "Resistance to *Bremia lactucae* (Bl)"Ad. 38 to ~~52-53~~: Resistance to *Bremia lactucae* (Bl), several isolates

1.	Pathogen	<i>Bremia lactucae</i>
2.	Quarantine status	no
3.	Host species	lettuce - <i>Lactuca sativa</i> L.
4.	Source of inoculum	GEVES ² (FR) or Naktuinbouw ³ (NL)
5.	Isolate	Bl: 16EU, 17EU, 20-27EU, 29-31EU, 33EU, 35EU, <u>36EU</u>
6.	Establishment isolate identity	test on differentials (see table below)
7.	Establishment pathogenicity	test on susceptible varieties
8.	Multiplication inoculum	
8.2	Multiplication variety	susceptible variety, for example Green Towers. For higher isolates, For isolates with a higher number than Bl: 16EU, a variety with defeated resistance may be preferable to keep the isolate fit.
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 3×10^4 - 1×10^5
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.

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11.	Observations	
11.1	Method	visual observation of sporulation and necrotic reaction to infection
11.2	Observation scale	<p>resistant:</p> <p>class 0 no sporulation, no necrosis</p> <p>class 1 no sporulation, necrosis present</p> <p>class 2 weak sporulation (much less than susceptible control) with necrosis</p> <p>class 3 weak sporulation (less than susceptible control and not evolving between second and third observation) with necrosis</p> <p>class 4 very sparse sporulation (not evolving between second and third observation) without necrosis</p> <p>susceptible:</p> <p>class 5 reduced sporulation (compared to susceptible control) without necrosis</p> <p>class 6 normal sporulation without necrosis</p>
11.3	Validation of test	<p>on standards</p> <p>in case of normal sporulation (same level as susceptible control) with necrosis another test on bigger plants or other substrate must be undertaken.</p>
12.	Interpretation of data in terms of UPOV characteristic states	<p>class 0, 1, 2, 3 and 4: resistant</p> <p>class 5 and 6: susceptible</p>
13.	Critical control points	<p>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.</p> <p>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.</p> <p>in case of use of nutritive solution on blotting paper, a fungicide can be added to avoid contamination by saprophytes.</p>

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/>. Pictures for the observation scale are also provided.

Isolates	Differentials	Green Towers	Dandle	R4T57D	UC Dm14	NunDm15	CGDm16	Colorado	F rRsal-1	Argelés	RYZ 2164	RYZ910457	Bedford	Balesta	Bartoli	Design	Kibrille
BI: 16EU	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
BI: 17EU	+	+	-	+	+	-	+	+	-	-	-	(+)	-	-	-	-	-
BI: 20EU	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
BI: 21EU	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-
BI: 22EU	+	-	+	+	+	-	+	-	-	-	-	-	+	-	-	-	-
BI: 23EU	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
BI: 24EU	+	-	+	-	-	+	+	-	+	-	-	-	-	-	-	(-)	-
BI: 25EU	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-
BI: 26EU	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-
BI: 27EU	+	+	+	+	+	-	+	-	+	+	-	(-)	+	-	-	-	-
BI: 29EU	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
BI: 30EU	+	-	+	+	+	-	+	-	+	+	-	-	-	-	-	+	-
BI: 31EU	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	+	-
BI: 33EU	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+
BI: 35EU	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+
BI: 36EU	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-

Resistance is indicated with – or (-), susceptibility is indicated with + or (+). The brackets indicate a lower and sometimes variable level of expression of the phenotype.

Proposed inclusion of characteristics from the Table of Characteristics in the Technical Questionnaire

5. The TWV is invited to consider the inclusion of the following characteristics in the TQ (characteristics for inclusion indicated in highlight and underline):

Char. No.	(*)	Characteristic Name	Comments
1	(*)	Seed: color	
6	(*)	<u>Leaf: number of divisions</u>	
11	(*)	Leaf: anthocyanin coloration	
12	(*)	<u>Leaf: hue of anthocyanin coloration</u>	
13		<u>Leaf: area covered by anthocyanin coloration</u>	
14	(*)	<u>Leaf: color</u>	
15	(*)	Leaf: intensity of green color	
34		<u>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Time of harvest maturity</u>	
35	(*)	Time of beginning of bolting	
38		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 16EU</u>	
39		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 17EU</u>	
40		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 20EU</u>	
41		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 21EU</u>	
42		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 22EU</u>	
43		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 23EU</u>	
44		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 24EU</u>	
45		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 25EU</u>	
46		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 26EU</u>	
47		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 27EU</u>	
48		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 29EU</u>	
49		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 30EU</u>	
50		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 31EU</u>	
51		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 33EU</u>	
52		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 35EU</u>	
53		<u>Resistance to <i>Bremia lactucae</i> (Bl) Isolate Bl: 36EU</u>	
54		<u>Resistance to Lettuce mosaic virus (LMV) Pathotype II</u>	
55		<u>Resistance to <i>Nasonovia ribisnigri</i> (Nr) Biotype Nr: 0</u>	
56		<u>Resistance to <i>Fusarium oxysporum</i> f. sp. <i>lactucae</i> (Fol) Race 1</u>	

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5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).

Characteristics	Example Varieties	Note
5.1 Seed: color (1)		
white	Verpia	1 []
yellow	Durango	2 []
brown	Oaklin	3 []
black	Kagraner Sommer 2	4 []
5.2 Leaf: number of divisions (6)		
<u>absent or very few</u>	<u>Fiorella, Lollo rossa</u>	<u>1 []</u>
<u>very few to few</u>		<u>2 []</u>
<u>few</u>	<u>Curletta, Rodagio</u>	<u>3 []</u>
<u>few to medium</u>		<u>4 []</u>
<u>medium</u>	<u>Ezabel, Jadigon</u>	<u>5 []</u>
<u>medium to many</u>		<u>6 []</u>
<u>many</u>	<u>Expedition, Multired 54</u>	<u>7 []</u>
<u>many to very many</u>		<u>8 []</u>
<u>very many</u>	<u>Excite, Ezfrill, Telex</u>	<u>9 []</u>
5.3 Leaf: anthocyanin coloration (11)		
absent or very weak	Clarion	1 []
very weak to weak		2 []
weak	Du bon jardinier	3 []
weak to medium		4 []
medium	Lollo rossa, Luana	5 []
medium to strong		6 []
strong	Merveille des quatre saisons	7 []
strong to very strong		8 []
very strong	Iride, Revolution	9 []
5.4 Leaf: hue of anthocyanin coloration (12)		
<u>reddish</u>	<u>Lollo rossa</u>	<u>1 []</u>
<u>purplish</u>	<u>Iride</u>	<u>2 []</u>
<u>brownish</u>	<u>Luana, Maravilla de Verano</u>	<u>3 []</u>

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Characteristics	Example Varieties	Note
5.5 (13) <u>Leaf: area covered by anthocyanin coloration</u>		
<u>very small</u>	<u>Steirer Krauthauptel</u>	1 []
<u>very small to small</u>		2 []
<u>small</u>	<u>Diablo</u>	3 []
<u>small to medium</u>		4 []
<u>medium</u>	<u>Luana</u>	5 []
<u>medium to large</u>		6 []
<u>large</u>	<u>Merveille des quatre saisons</u>	7 []
<u>large to very large</u>		8 []
<u>very large</u>	<u>Bijou, Revolution</u>	9 []
5.6 (14) <u>Leaf: color</u>		
<u>green</u>	<u>Verpia</u>	1 []
<u>yellowish green</u>	<u>Dorée de printemps</u>	2 []
<u>greyish green</u>	<u>Celtuce, Du bon jardinier</u>	3 []
5.7 (15) <u>Leaf: intensity of green color</u>		
<u>very light</u>		1 []
<u>very light to light</u>		2 []
<u>light</u>	<u>Blonde maraîchère, Lollo Bionda</u>	3 []
<u>light to medium</u>		4 []
<u>medium</u>	<u>Aquarel, Clarion</u>	5 []
<u>medium to dark</u>		6 []
<u>dark</u>	<u>Expedition, Verpia</u>	7 []
<u>dark to very dark</u>		8 []
<u>very dark</u>	<u>Pascal, Verdatrix</u>	9 []
5.8 (34) <u>Only varieties with Plant: degree of overlapping of upper part of leaves: medium or strong: Time of harvest maturity</u>		
<u>very early</u>	<u>Gotte jaune d'or</u>	1 []
<u>very early to early</u>		2 []
<u>early</u>	<u>Pantlika, Sucrine</u>	3 []
<u>early to medium</u>		4 []
<u>medium</u>	<u>Clarion</u>	5 []
<u>medium to late</u>		6 []
<u>late</u>	<u>Blonde maraîchère, Calmar</u>	7 []
<u>late to very late</u>		8 []
<u>very late</u>	<u>El Toro, Pinokkio</u>	9 []

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Characteristics	Example Varieties	Note
5.9 Time of beginning of bolting (35)		
very early	Blonde à couper améliorée	1 []
very early to early		2 []
early	Gotte à graine blanche	3 []
early to medium		4 []
medium	Pantlika	5 []
medium to late		6 []
late	Hilde II	7 []
late to very late		8 []
very late	Erika, Roxette	9 []
5.10 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 16EU (38)		
<u>absent</u>	<u>Green Towers</u>	1 []
<u>present</u>	<u>Argelès</u>	9 []
<u>not tested</u>		[]
5.11 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 17EU (39)		
<u>absent</u>	<u>Green Towers</u>	1 []
<u>present</u>	<u>Argelès</u>	9 []
<u>not tested</u>		[]
5.12 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 20EU (40)		
<u>absent</u>	<u>Green Towers</u>	1 []
<u>present</u>	<u>FrRsal-1</u>	9 []
<u>not tested</u>		[]
5.13 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 21EU (41)		
<u>absent</u>	<u>Green Towers</u>	1 []
<u>present</u>	<u>Argelès, Colorado</u>	9 []
<u>not tested</u>		[]
5.14 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 22EU (42)		
<u>absent</u>	<u>Green Towers</u>	1 []
<u>present</u>	<u>FrRsal-1</u>	9 []
<u>not tested</u>		[]
5.15 Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 23EU (43)		
<u>absent</u>	<u>Green Towers</u>	1 []
<u>present</u>	<u>Colorado</u>	9 []
<u>not tested</u>		[]

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Characteristics	Example Varieties	Note
5.16 (44) <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 24EU</u>		
<u>absent</u>	<u>Argelès, Colorado</u>	<u>1 []</u>
<u>present</u>	<u>Dandie, NunDm15, UCDm14</u>	<u>9 []</u>
<u>not tested</u>		<u>[]</u>
5.17 (45) <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 25EU</u>		
<u>absent</u>	<u>Colorado</u>	<u>1 []</u>
<u>present</u>	<u>Argelès</u>	<u>9 []</u>
<u>not tested</u>		<u>[]</u>
5.18 (46) <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 26EU</u>		
<u>absent</u>	<u>Colorado</u>	<u>1 []</u>
<u>present</u>	<u>Balesta, Bedford</u>	<u>9 []</u>
<u>not tested</u>		<u>[]</u>
5.19 (47) <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 27EU</u>		
<u>absent</u>	<u>Balesta, Colorado</u>	<u>1 []</u>
<u>present</u>	<u>FrRsal-1</u>	<u>9 []</u>
<u>not tested</u>		<u>[]</u>
5.20 (48) <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 29EU</u>		
<u>absent</u>	<u>Argelès</u>	<u>1 []</u>
<u>present</u>	<u>Balesta</u>	<u>9 []</u>
<u>not tested</u>		<u>[]</u>
5.21 (49) <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 30EU</u>		
<u>absent</u>	<u>Argelès, Colorado</u>	<u>1 []</u>
<u>present</u>	<u>Balesta</u>	<u>9 []</u>
<u>not tested</u>		<u>[]</u>
5.22 (50) <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 31EU</u>		
<u>absent</u>	<u>Colorado, RYZ910457</u>	<u>1 []</u>
<u>present</u>	<u>Argelès, Balesta</u>	<u>9 []</u>
<u>not tested</u>		<u>[]</u>
5.23 (51) <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 33EU</u>		
<u>absent</u>	<u>Kibrille, RYZ2164</u>	<u>1 []</u>
<u>present</u>	<u>RYZ910457</u>	<u>9 []</u>
<u>not tested</u>		<u>[]</u>

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Characteristics	Example Varieties	Note
<u>5.24</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 35EU</u> <u>(52)</u>		
<u>absent</u>	<u>Design, Kibrille</u>	1 []
<u>present</u>	<u>Bartoli</u>	9 []
<u>not tested</u>		[]
<u>5.25</u> <u>Resistance to <i>Bremia lactucae</i> (BI) Isolate BI: 36EU</u> <u>(53)</u>		
<u>absent</u>	<u>Bartoli, RYZ2164</u>	1 []
<u>present</u>	<u>Design, Kibrille</u>	9 []
<u>not tested</u>		[]
<u>5.26</u> <u>Resistance to <i>Lettuce mosaic virus</i> (LMV) Pathotype II</u> <u>(54)</u>		
<u>absent</u>	<u>Bijou, Hilde II, Sprinter, Sucrine</u>	1 []
<u>present</u>	<u>Capitan, Corsica</u>	9 []
<u>not tested</u>		[]
<u>5.27</u> <u>Resistance to <i>Nasonovia ribisnigri</i> (Nr) Biotype Nr: 0</u> <u>(55)</u>		
<u>absent</u>	<u>Abel, Green Towers, Nadine</u>	1 []
<u>present</u>	<u>Barcelona, Bedford, Dynamite, Silvinas</u>	9 []
<u>not tested</u>		[]
<u>5.28</u> <u>Resistance to <i>Fusarium oxysporum</i> f.sp. <i>lactucae</i> (Fol) Race 1</u> <u>(56)</u>		
<u>susceptible</u>	<u>Cobham Green, Patriot</u>	1 []
<u>moderately resistant</u>	<u>Affic, Fuzila, Natexis</u>	2 []
<u>highly resistant</u>	<u>Costa Rica No. 4, Romasol</u>	3 []
<u>not tested</u>		[]

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