



## **PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS**

*Lactuca sativa* L.

**LETTUCE**

UPOV Code: LACTU\_SAT

**Adopted on 16/02/2011**

**Entered into force on 01/01/2011**

## **I SUBJECT OF THE PROTOCOL**

The protocol describes the technical procedures to be followed in order to meet the Council Regulation (EC) No. 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on general UPOV Document TG/1/3 and UPOV Guideline TG/13/10 dated 05/04/2006 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies for all varieties of *Lactuca sativa* L.

## **II SUBMISSION OF SEED AND OTHER PLANT MATERIAL**

### 1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of

- the closing date for the receipt of plant material;
- the minimum amount and quality of plant material required;
- the Examination Office to which material is to be sent.

A sub-sample of the material submitted for test will be held in the variety collection as the definitive sample of the candidate variety.

The applicant is responsible for ensuring compliance with any customs and plant health requirements.

### 2. Final dates for receipt of documentation and material by the Examination Office

The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen.

The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO whether acceptable plant material has been received or not. However if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

### 3. Plant material requirements

The current quality and quantity requirements as well as the final dates for submission of the plant material are available on the CPVO website ([www.cpvo.europa.eu](http://www.cpvo.europa.eu)) and are published in the CPVO gazette 'S2'.

Quality of seeds: Should not be less than the standards laid down for certified seed in Annex II of Council Directive 2002/55/EC.

Seed treatment: The plant material must not have undergone any treatment unless the CPVO and the Examination Office allow or request such treatment. If it has been treated, full details of the treatment must be given.

Special requirement: -

Labelling of sample:

- Species
- File number of the application allocated by the CPVO
- Breeder's reference
- Examination office's reference (if known)
- Name of applicant
- The phrase "On request of the CPVO"

### III CONDUCT OF TESTS

#### 1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation (EC) No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;
- varieties protected in other UPOV Member States;
- any other variety in common knowledge.

The composition of the variety collection in each Examination Office depends on the environmental conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material, Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

#### 2. Material to be examined

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the variety collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate the work with other Offices involved in DUS testing of lettuce. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

#### 3. Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the Annex I. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted. In the latter case, the CPVO should be informed. In addition the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation (EC) No. 1239/95, to insert additional characteristics and their expression in respect of a variety.

#### 4. Grouping of varieties

The varieties and candidates to be compared will be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups.

In the first place, the collection should be divided according to the following growth types (for further information see chapter on explanations and methods):

	Examples
1. Butterhead lettuce	Clarion, Merveille des quatre saisons, Verpia
2. Crisphead lettuce	Blonde de Paris (Batavia), Calmar, Saladin (Iceberg)
3. Cos (Roman) lettuce	Blonde maraîchère (Roman types)
4. "Grasse" or Latin lettuce	Bibb, Sucrine
5. Cutting/Gathering lettuce	Frisé d'Amérique, Lollo rossa, Oakleaf, Salad Bowl
6. Stem lettuce	Celtuce

The characteristics used for grouping could be the following:

- a) Seed: colour (characteristic 1)
- b) Leaf: anthocyanin coloration (characteristic 18)
- c) Time of beginning of bolting under long day conditions (characteristic 33)
- d) Resistance to downy mildew (*Bremia lactucae*) Isolate B1:16 (characteristic 37.7)

5. Trial designs and growing conditions

The minimum duration of tests will normally be two independent growing cycles. Tests will be carried out under conditions ensuring normal growth. The size of the plots will be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing period.

The test design is as follows

Each test should include 60 plants divided between two or more replicates.

All observations determined by measurement or counting should be made on 20 plants or parts of 20 plants.

6. Special tests

In accordance with Article 83(3) of Council Regulation (EC) No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate variety has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

7. Standards for decisions

a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of Article 7 of Council Regulation (EC) No. 2100/94.

b) **Uniformity**

A candidate will be considered to be sufficiently uniform if the number of off-types does not exceed the number of plants as indicated in the table below. A population standard of 1% and an acceptance probability of 95% should be applied.

Table of maximum numbers of off-types allowed for uniformity standards.

Number of plants	off-types allowed
36-82	2

c) **Stability**

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity.

**IV REPORTING OF RESULTS**

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two growing periods but in some cases three growing periods may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive from the Examination Office interim reports and final reports by the date agreed between the CPVO and the Examination Office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

**V LIAISON WITH THE APPLICANT**

If problems arise during the course of the test the CPVO should be informed immediately so that the information can be passed on to the applicant. Subject to prior agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

The interim report as well as the final report shall be sent by the Examination Office to the CPVO.

**VI - ENTRY INTO FORCE**

The present protocol enters into force on 01/01/2011. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the new TP. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for the submission of plant material for the first growing period.

In cases where the CPVO requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process of being carried out at the moment of the request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

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## ANNEXES TO FOLLOW

ANNEX I	PAGE
Table of characteristics .....	7
Explanations and methods .....	17

### Legend:

Note: For the CPVO numbered characteristics, all characteristics in the table are compulsory; notwithstanding, in the case of disease resistance characteristics, only those resistances marked with an asterisk (\*) in the CPVO column are compulsory. The asterisks in the UPOV numbered characteristics are there for information purposes and denote those characteristics which should always be observed when a UPOV guideline is utilised.

For varieties testing resistance "present" (9) to *Bremia lactucae* Isolate BI 16 (characteristic 37.7), it is compulsory to test Isolates BI: 20, BI: 21, BI: 22, BI: 23, BI: 24, BI: 25 and BI: 26 (characteristics 37.9 – 37.16, hence with marked [\*])

In general for the assessment of resistance characteristics, the facilities of other Examination Offices or specialised institutions might be used, subject to previous arrangements. Some characteristics may be discarded: if there are already phytosanitary restrictions.

(+) See explanations on the Table of characteristics

(a) – (c) See explanations on the table of characteristics

### Types of expression of characteristics:

QL – Qualitative characteristic

QN – Quantitative characteristic

PQ – Pseudo-qualitative characteristic

### Type of observation of characteristics:

MG – Single measurement of a group of plants or parts of plants

MS – Measurement of a number of individual plants or parts of plants

VG – Visual assessment by a single observation of a group of plants or parts of plants

VS – Visual assessment by observation of individual plants or parts of plants

When a method of observation is attributed to a certain characteristic, the first differentiation is made depending if the action taken is a visual observation (V) or a measurement (M).

The second differentiation deals with the number of observations the expert attributes to each variety, thus the attribution of either G or S.

If a single observation of a group consisting of an undefined number of individual plants is appropriate to assess the expression of a variety, we talk about a visual observation or a measurement made on a group of plants, thus we attribute the letter G (either VG or MG). If the expert makes more than one observation on that group of plants, the decisive part is that we have at the end only one data entry per variety which means that we have to deal with G (e.g. measurement of plant length on a plot – MG, visual observation of green colour of leaves on a plot – VG).

If it is necessary to observe a number of individual plants to assess the expression of a variety, we should attribute the letter S (thus either VS or MS). Single plant data entries are kept per variety for further calculations like the variety mean (e.g. measurement of length of ears – MS, visual observation of growth habit of single plants in grasses – VS). The number of individual plants to be observed in such cases is stated in section III.5.

Literature .....	28
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## ANNEX II

Technical Questionnaire

## ANNEX I

TABLE OF CHARACTERISTICS TO BE USED IN DUS TESTS AND PREPARATION OF DESCRIPTIONS

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note
1.	1.	VG	<b>Seed: colour</b>		
	(*)		white	Verpia	1
	QL		yellow	Durango	2
G			black	Kagraner Sommer	3
2. (+)	2. (+)	VG	<b>Seedling: anthocyanin coloration</b>		
	(*)		absent	Verpia	1
	QL		present	Pirat	9
3. (a)	5. (a)	VG	<b>Leaf: attitude at 10-12 leaf stage</b>		
	(a)		erect	Baby Star, Romance	1
	QN		semi-erect	Great Lakes 118, Soraya	3
			prostrate	Unicum, Vanguard 75	5
4. (+) (a)	6. (+) (a)	VG	<b>Leaf blade: division (at 10-12 leaf stage)</b>		
	(+)		entire	Fiorella, Sunrise	1
	(a)		lobed	A couper à feuille de chêne blonde à graine noire, Salad Bowl	2
	PQ		divided	Lagon, Monet	3
5. (a)	7. (*) (a) (a) (a)	VG	<b>Plant: diameter</b>		
	(*)		very small	Pavane, Tom Thumb	1
	(a)		small	Bastion, Gotte à graine blanche	3
	QN		medium	Clarion, Verpia	5
			large	Great Lakes 659, Musette	7
			very large	El Toro, Yuma	9

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note	
6.	8.	VG	<b>Plant: head formation</b>			
	(*)		no head	Blonde à couper améliorée, Lollo rossa	1	
	(a)		open head	Manfred, Monet	2	
	PQ		closed head (overlapping)	Kelvin, Sunrise	3	
7.	9.	VG	<b><u>Varieties with closed head formation only: Head: degree of overlapping of upper part of leaves</u></b>			
			(a)	very weak	Colorado	1
				weak	Danilla, Novita	3
				medium	Augusta, Fiorella	5
				strong	Master, Minas	7
				very strong	Kelvin, Roxette	9
8.	10.	VG	<b>Head: density</b>			
			(a)	very loose	Ninja	1
				loose	Danilla, Nanda	3
				medium	Blonde maraîchère	5
				dense	Hilde II, Kelvin	7
				very dense	Musette, Toronto	9
9.	11.	VG	<b>Head: size</b>			
			(a)	very small	Tom Thumb	1
				small	Bastion, Gotte à graine blanche	3
				medium	Fiorella, Soraya	5
				large	Great Lakes 659, Musette	7
				very large	Blonde maraîchère, El Toro	9
10.	12.	VG	<b><u>Butterhead type varieties in glasshouse only: Head: closing of base</u></b>			
			(a)	weak	Passe Partout	3
				medium	Carmelita	5
				strong	Dustin, Manfred	7



CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note
<b>11.</b>	<b>13.</b>	<b>VG</b>	<b>Head: shape in longitudinal section</b>		
(+)	(+)		narrow elliptic	Verte maraichère	1
(a)	(a)		broad elliptic	Amadeus, Sucrine	2
	(*)		circular	Passe Partout, Verpia	3
	PQ		transverse broad elliptic		4
<b>12.</b>	<b>14.</b>	<b>VG</b>	<b>Leaf: thickness</b>		
(a)	(a)		thin	Raisa, Royal Red	3
	QN		medium	Dustin, Sunrise	5
			thick	Frisée de Beauregard	7
<b>13.</b>	<b>15.</b>	<b>VG</b>	<b>Leaf: attitude at harvest maturity (outer leaves from head lettuce or adult leaves from cutting and stem lettuce)</b>		
(a)	(a)		erect	Feria, Riva	1
	QN		semi-erect	Amelia, Toronto	3
			horizontal	Chambery, Divina	5
<b>14.</b>	<b>16.</b>	<b>VG</b>	<b>Leaf: shape</b>		
(+)	(+)		narrow elliptic	Riva, Verte maraichère	1
(a)	(a)		medium elliptic	Angela, Xanadu	2
	(*)		broad elliptic	Amadeus, Amelia	3
	PQ		circular	Elsa, Sunrise, Verpia	4
			transverse broad elliptic	Commodore, Fiorella	5
			transverse narrow elliptic	Elvira, Madison	6
			obovate	Raisa, Toronto	7
			broad obtrullate	Delicato, Monet	8
			triangular	Deer Tongue	9

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note
15.	17.	VG	<b>Leaf: shape of tip</b>		
	(a)		acute	Celtuce, Deer Tongue, Karola, Temptra	1
	PQ		obtuse	Chicon des Charentes, Grise maraichère	2
rounded		Blonde Maraichère, Maserati	3		
16.	18.	VG	<b>Leaf: hue of green colour of outer leaves</b>		
	(+)		absent	Donatello, Verpia	1
	(a)		yellowish	Dorée de printemps	2
	(*)		greyish	Celtuce, Du bon jardinier	3
	PQ	reddish	Lollo rossa, Revolution, Rosa (see also Ad. 16)	4	
17.	19.	VG	<b>Leaf: intensity of colour of outer leaves</b>		
	(+)		very light	(see also Ad. 16)	1
	(a)		light	(see also Ad. 16)	3
	(*)		medium	(see also Ad. 16)	5
	QN		dark	(see also Ad. 16)	7
very dark		(see also Ad. 16)	9		
18.	20. (*)	VG	<b>Leaf: anthocyanin coloration</b>		
	(a)		absent	Fiorella, Sunrise	1
	G		QL	present	Commodore, Pirat
19.	21.	VG	<b>Leaf: intensity of anthocyanin coloration</b>		
	(*)		very weak	Chicon de Charentes, Muranta, Rumina	1
	(a)		weak	Du bon jardinier	3
			QN	medium	Trocadéro à graine noire
	strong		Amandine, Merveille des quatre saisons	7	
very strong	Little Leprechaun, Revolution	9			

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note		
20.	21.	VG	<b>Leaf: distribution of anthocyanin</b>				
			(+)	localised	Muranta, Rumina, Bughatti, Murano	1	
	(a)	QL	entire	Delicato, Liberty, Loretta, Bijou	2		
21.	23.	VG	<b>Leaf: kind of anthocyanin distribution</b>				
			(a)	(a)	diffused only	Amandine, Pirat, Sanguine	1
				QL	in spots only	Passion blonde à graine blanche, Unicum	2
			diffused and in spots	Lovina, Rougette du Midi	3		
22.	24.	VG	<b>Leaf: glossiness of upper side</b>				
			(a)	(a)	absent or very weak	Divina, Du bon jardinier	1
				QN	weak	Elsa, Fiorella	3
					medium	Feria, Sunrise	5
			strong	Ibis, Noisette	7		
23.	25.	VG	<b>Leaf: blistering</b>				
				(*)	absent or very weak	Donia, Frillblond	1
			(a)	(a)	weak	Fiorella, Minas	3
				QN	medium	Commodore	5
					strong	Blonde de Paris, Smile	7
			very strong	Blonde de Doulon	9		
24.	26.	VG	<b>Leaf: size of blisters</b>				
			(a)	(a)	small	Dorée de printemps	3
				QN	medium	Dustin, Sunrise	5
			large	Fiorella, Massilia	7		

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note
25.	27.	VG	<b>Leaf blade: degree of undulation of margin</b>		
(a)	(a)		absent or very weak	Dustin, Manfred	1
	QN		weak	Commodore, Sunrise	3
			medium	Noisette, Pentared	5
			strong	Calmar, Invicta	7
			very strong	Lollo rossa, Madison	9
26.	28.	VG	<b>Leaf blade: incisions of margin on apical part</b>		
(a)	(a)		absent	Verpia	1
	QL		present	Calmar, Gloire du Dauphiné, Unicum	9
27.	29.	VG	<b>Leaf blade: depth of incisions of margin on apical part</b>		
	(*)		shallow	Pentared, Unicum	3
(a)	(a)		medium	Ithaca Great Lakes	5
	QN		deep	Lagon, Monet	7
28.	30.	VG	<b>Leaf blade: density of incisions on margin on apical part</b>		
(a)	(a)		sparse	Maravilla de Verano	3
	QN		medium	Calmar, De Pierre Benite	5
			dense	Grand Rapids, Ithaca Great Lakes	7
			very dense	Locarno, Madison	9
29.	31.	VG	<b><u>Varieties with shallow incisions on margin on apical part only:</u> Leaf blade: type of incisions on apical part</b>		
(a)	(a)		sinuate	Gloire du Dauphiné	1
	QL		dentate	Calmar	2
30.	32.	VG	<b>Leaf blade: venation</b>		
(a)	(a)		not flabellate	Donatella, Verpia, Xanadu	1
	QL		flabellate	Gloire du Dauphiné, Locarno, Monet	2

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note	
31.	33.	VG	<b>Axillary sprouting</b>			
			absent or very weak	Valmaine	1	
			weak	Aprilia, Sunrise	3	
			medium		5	
			strong	Riva	7	
			very strong	Doncella	9	
32.	34.	MG	<b>Time of harvest maturity</b>			
			very early	Blonde à couper améliorée	1	
			early	Attraction	3	
			medium	Newton	5	
			late	Calmar	7	
			very late	El Toro	9	
33.	35.	MG	<b>Time of beginning of bolting under long day conditions</b>			
			(*)	very early	Blonde à couper améliorée	1
			QN	early	Gotte à graine blanche	3
				medium	Carelia	5
				late	Hilde II	7
G	very late	Erika, Kinemontepas, Rex	9			
34.	36.	VG/MG	<b>Plant: height (flowering plant)</b>			
			QN	short	Gotte à graine blanche	3
				medium	Samourai	5
tall	Danilla, Hilde II	7				
35.	37.	VG	<b>Plant: fasciation (at flowering stage)</b>			
			QL	absent	Calmar, Romance	1
		present		Gotte jaune d'or	9	

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note		
36.	38.	VG	<b>Plant: intensity of fasciation (flowering plant)</b>				
				QN	very weak	Gotte à graine blanche	1
					weak	Verte maraichère	3
					medium	Amadeus	5
					strong	Gotte jaune d'or	7
			very strong	Chicon des Charentes	9		
37. (+) (a) (b)	39. (+) (a) (b)	VG QL	<b>Resistance to downy mildew (<i>Bremia lactucae</i>)</b>				
				37.1	39.1	<b>Isolate BI: 2</b>	
			absent	Cobham Green, Green Towers	1		
			present	Ninja	9		
37.2	39.2		<b>Isolate BI: 5</b>				
				absent	Cobham Green, Green Towers	1	
			present	Sabine	9		
37.3	39.3		<b>Isolate BI: 7</b>				
				absent	Cobham Green, Green Towers	1	
			present	Valmaine	9		
37.4	39.4		<b>Isolate BI: 12</b>				
				absent	Cobham Green, Green Towers	1	
			present	Dandie, UCdM2	9		
37.5	39.5		<b>Isolate BI: 14</b>				
				absent	Cobham Green, Green Towers	1	
			present	Colorado, Ninja	9		
37.6	39.6		<b>Isolate BI: 15</b>				
				absent	Cobham Green, Green Towers	1	
			present	Colorado, Sabine	9		

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note
<b>37.7</b>	<b>39.7</b>		<b>Isolate BI: 16</b>		
(*)	(*)		absent	Cobham Green, Green Towers	1
G			present	Argelès, Ninja	9
<b>37.8</b>	<b>39.8</b>		<b>Isolate BI: 17</b>		
			absent	Cobham Green, Green Towers	1
			present	Argelès, Ninja	9
<b>37.9</b>	<b>39.9</b>		<b>Isolate BI: 18</b>		
			absent	Cobham Green, Green Towers	1
			present	Argelès, Ninja	9
<b>37.10</b>	<b>39.10</b>		<b>Isolate BI: 20</b>		
(*)			absent	Cobham Green, Green Towers	1
			present	Argelès, Ninja	9
<b>37.11</b>	<b>39.11</b>		<b>Isolate BI: 21</b>		
(*)			absent	Cobham Green, Green Towers	1
			present	Argelès, Ninja	9
<b>37.12</b>	<b>39.12</b>		<b>Isolate BI: 22</b>		
(*)			absent	Cobham Green, Green Towers	1
			present	Discovery, Ninja	9
<b>37.13</b>	<b>39.13</b>		<b>Isolate BI: 23</b>		
(*)			absent	Cobham Green, Green Towers	1
			present	Colorado, Discovery, Ninja	9
<b>37.14</b>	<b>39.14</b>		<b>Isolate BI: 24</b>		
(*)			absent	Argeles, Colorado	1
			present	Dandie, Nun Dm15, UC DM14	9
<b>37.15</b>	<b>39.15</b>		<b>Isolate BI: 25</b>		
(*)			absent	Colorado, Pennlake	1
			present	Argèles, Ninja	9

CPVO No.	UPOV No.	Stage	Characteristics	Examples	Note
37.16 (* )	39.16		<b>Isolate BI: 26</b>		
			absent	Colorado, Discovery	1
			present	Balesta, Bedford	9
37.17	39.17		<b>Isolate BI: 27</b>		
			absent	Balesta, Green Towers	1
			present	Bedford	9
38. (+ )	40. (+ ) QL	VG	<b>Resistance to lettuce mosaic virus (LMV)</b>  <b>Strain Ls 1</b>		
			absent	Hilde II, Salvina	1
			present	Corsica	9
39. (+ )	QL	VG	<b>Resistance to <i>Nasonovia ribisnigri</i> biotype Nr: 0</b>		
			absent	Abel, Nadine, Green Towers	1
			present	Barcelona, Dynamite, Silvinas	9



## EXPLANATIONS AND METHODS

### Key to Lettuce Types (under Section 5.3)

Cultivated lettuce varieties (vegetables) can be grouped into the following growth types:

(1) Butterhead Lettuce

Heading or with a tightly filled heart, tender leaves with a clear midrib; head shape ranging from broad elliptic to transverse elliptic.

(2) Crisphead Lettuce (including the Iceberg, Batavia and Maravilla types)

Weak to very strong heading, rather thin to very thick and tough leaves, no clear midrib but with flabellate venation.

Iceberg types (like Calmar and Saladin) are mainly thick and tough-leaved, predominantly green and grey-green, leaf margin hardly to rather strongly incised.

Batavia types are generally medium thick-leaved and with rather strongly blistered leaves, predominantly yellowish or medium green; under cold conditions not always clearly heading.

Maravilla types have rather thick and tough leaves, only slightly or not blistered.

(3) Cos Lettuce (Roman Lettuce)

Heading or semi-heading, elongated and rather tough leaves with a clear midrib, head shape in longitudinal section narrow elliptic.

(4) "Grasse" or Latin Lettuce (sometimes included under Cos Lettuce)

Heading or semi-heading, tough thick leaves with clear midrib, head shape broad elliptic to slightly obovate. Some types only have a tightly filled heart, others are more similar to a short Cos Lettuce. Suitable for semi-arid conditions.

(5) Cutting or Gathering Lettuce

Rather heterogeneous group ranging from non-heading butterhead-like, non-heading Batavia-like, non-heading crisp types to Oakleaf and Catalogna (lobed) types with deeply dissected leaves (Monet) and types with strongly undulated leaf margin (Lollo). Varieties partly with a clear midrib and partly with flabellate venation of the leaves. Common characteristic: loose-leaved rosette.

(6) Stem Lettuce

Forms a fleshy stem before bolting, at least under (semi-) short day conditions; leaves are mainly tough and have a clear midrib. Leaves and/or stem are consumed.

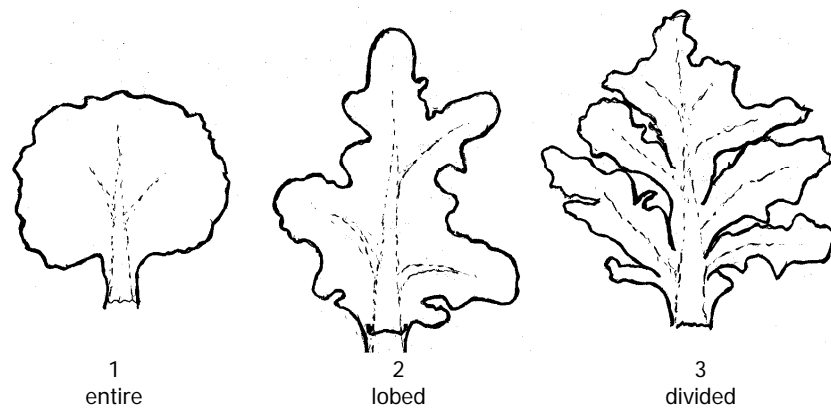
### Explanations covering several characteristics

- (a) Plant, head, leaf, leaf blade: Observations on the plant, head, leaf and leaf blade should be made at harvest maturity.
- (b) Disease resistance: When disease resistance characteristics are used for assessing distinctness, uniformity and stability, records should be taken under conditions of controlled infection with a defined pathotypes.
- (c) Resistance to downy mildew: Each race should be tested separately and the results should also be indicated separately.

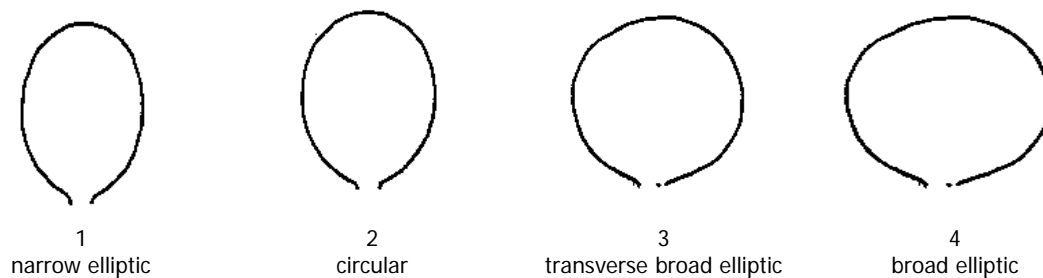
#### Ad. 2: Seedling: anthocyanin coloration

This characteristic can easily be observed by keeping the remaining seedlings after pricking out in the seeding tray without watering and under cold(er) conditions. Within two or three days all seedlings of varieties with anthocyanin will show this characteristic.

#### Ad. 5: Leaf blade: division



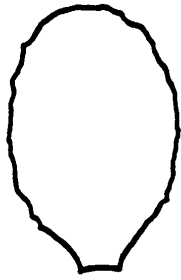
#### Ad. 11: Head: shape in longitudinal section



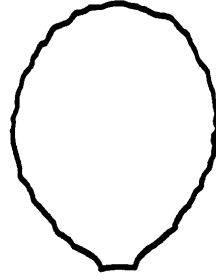
Ad. 14: Leaf: shape



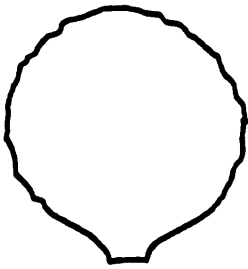
1  
narrow elliptic



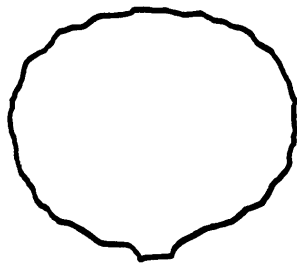
2  
medium elliptic



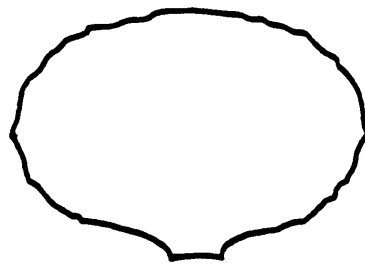
3  
broad elliptic



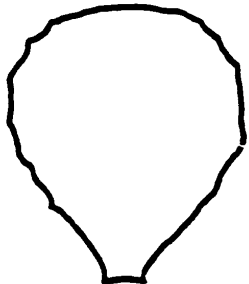
4  
circular



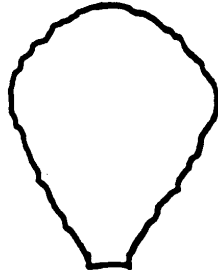
5  
transverse broad elliptic



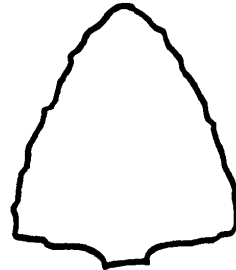
6  
transverse narrow elliptic



7  
obovate



8  
broad obtrullate



9  
triangular

Ad. 16: Leaf: hue of green colour of outer leaves

Ad. 17: Leaf: intensity of colour of outer leaves

Intensity of colour (Ch. 19)	Hue of green colour (Ch. 18)			
	1 absent	2 yellowish	3 greyish	4 reddish
1 very light	Krizet	Marbello Black Seeded Simpson	Hohlblättriger Butter	
3 light	Blonde maraichère, Mondial, Reskia	Blondine (= Viktoria), Locarno, Pia	Celtuce, Kinemontepas, Natina	Brauner Troztkopf, Maravilla de Verano
5 medium	Florian, Frillblond, Sunrise, Têtue de Nimes	Australische Gele, Dorée de printemps, Gotte jaune d'or	Clarion, Du bon jardinier, Durango, Kelvin	Lollo rossa, Pirat, Prizehead (= Frisée d'Amérique)
7 dark	Baby Star, Donatello, Verpia, Waldemann Dark Green	Batavia, Chicon	Chou de Naples (= Webb's Wonderful), Galaxy, Toledo	Merveille des quatre saisons, Rosa, Rouge d'Hiver
9 very dark	Pavane		(Sudia)	Liberty, Malibu, Pentared, Revolution

Ad. 20: Leaf: distribution of anthocyanin

Varieties with anthocyanin distribution "entire": The sight of the plant on the field must be completely anthocyanised. However, the leaves may have a certain wide green region on the basal part which is visible when splitting them off.

Ad. 37: Resistance to downy mildew (*Bremia lactucae*)

Availability of *Bremia* isolates and differentials

There are two centres, the "Station nationale d'essais de semences" (SNES) in France and the Naktuinbouw in the Netherlands, which would verify and test the isolates as defined and denominated by IBEB (the International Bremia Evaluation Board) 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

Naktuinbouw  
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

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 BI: 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: At least 30 separate plants of each variety should be tested to establish the uniformity.
- (d) Temperature: Incubation of inoculated seedlings or leaf discs should be at 15-18°C.
- (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 day, or two of these three time. The time of maximum sporulation should occur in this period.
- (h) Recording method and interpretation of the data. The following symptoms may be recorded:
1. Abundant white sporulation on both sides of the cotyledon; this is translated in the table as (+)
  2. Normal sporulation only on the lower side of the cotyledons; this is translated in the table as (+).
  3. Normal sporulation only on the lower side of the cotyledons combined with necrotic spots; this is translated in the table as (+).
  4. Sparse sporulation on the lower side of the cotyledons combined with necrosis; this is translated in the table as (-).
  5. Necrotic pinpoints; this is translated in the table as (-).
  6. No symptoms; this is translated in the table as (-).

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

Class 1, 2 and 3; susceptible

Class 4, 5 and 6; resistant

[Note on the table:

Compared to the former table PIVT1309, LSE/18 and Ls102 are replaced by Nun Dm15, CGDm16 and NunDm17 respectively. They have the same resistance pattern, but a better germination.]

More detailed information about the use of the table can be found in the relevant literature.

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	Argelés	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		+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	-	+	-

Ad. 38: Resistance to Lettuce Mosaic Virus (LMV)

Maintenance of strains

Maintenance: After 15-20 days of incubation infected tissue should be sliced and desiccated over calcium chloride and stored at 4°C. Infectivity may last 1 to 3 years. Contamination can be avoided in this way.

Multiplication: Pre-multiplication of the virus on a susceptible variety (e.g. Hilde or Trocadero) prior to testing under normal conditions. Only virus-free seed samples should be used for this purpose.

Execution of test

Growth stage of plants: First inoculation at 2 to 3 leaves stage.

Temperature: Constant temperature of 16°C during night (N) and of 22°C during day (D) or, alternatively, temperature of 20°C N, 25°C D during 5 days after inoculation followed by 12°C N and 18°C D.

Light conditions: From emergence: 16 hours per day, at least 15,000 Lux.

Preparation of inoculum: Young leaves of diseased lettuce plants showing clear LMV symptoms (after 15-25 days of incubation) should be ground (1 g fresh leaves per 4 ml) in a mortar adding a 0.03 M Na<sub>2</sub>HPO<sub>4</sub>-buffer containing 0.2% DIECA<sup>(\*)</sup>. Prior to inoculation 75 mg/ml carborundum and 75 mg/ml activated charcoal should be added.

(\*) Composition of buffer: per 100 ml: 1.07 g Na<sub>2</sub>HPO<sub>4</sub> 12H<sub>2</sub>O, 0.2 g DIECA

Method of inoculation: Mechanical inoculation by rubbing on the two first leaves, followed by a second inoculation 2-3 days afterwards. The inoculum is kept in an ice bucket during inoculation.

Duration of test: - From sowing to inoculation: about 2 weeks  
- From inoculation to reading: about 2 to 3 weeks; first reading after 15 days

Number of plants tested: 20 plants and 2 repetitions

*Remarks:*

Strains: Other strains of LMV have been isolated in Europe (France, Greece, Spain) by Dinant and Lot (1992), Plant Pathology 41:528-542. The naming of the strains is not yet internationally accepted; but names of pathotypes have been proposed (Pink, Lot and Johnson (1992), Euphytica 63:169-174).

Symptoms (under test conditions): The expression of the symptoms depends on the strains and the lettuce genotypes. For the old Ls-1 strain used for testing the 'Gallega'-gene, the typical reactions can be summarized as follows:

Butterhead cultivars show essentially vein clearing and mosaic;

Crisp or Iceberg cultivars show chlorosis along the veins and faint mosaic;

Cos cultivars show reduced growth of the inner leaves and blistering;

In red varieties symptoms are particularly difficult to observe.

Ad. 39: Resistance to *Nasonovia ribisnigri* biotype Nr:0

Maintenance of biotype	<i>Nasonovia ribisnigri</i> is a leaf aphid and may be maintained alive on susceptible lettuce plants in aphid-proof chambers or tents in a glasshouse. <i>N. ribisnigri</i> 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.
Identity check	The aphids' body size is 1.5-2.5 mm. The body has 7 dark spots. The ends of the legs are black.
Multiplication	on susceptible variety at 20-22°C for 10-14 d. Aphids are shaken off into Petri dish
Sowing	12°C for germination and early growth; plant distance >5 cm
Plant stage at inoculation	15 d
Inoculation method	careful transfer of 5 aphids per plant using a fine paintbrush
Temperature	20-22°C
First observation	10 days after inoculation
Second observation	daily check whether newborn aphids are mature (= red)
End of test	max. 15 d after inoculation
Observation at end of test	number of mature (= red) aphids on each plant
Number of plants tested	28
Scale for observations	0 - no aphids 1 - 1-5 aphids per plant 2 - 6-10 aphids per plant 3 - > 10 aphids per plant
Interpretation of data	0 or 1 - Resistant 2 - Undecided 3 - Susceptible
Remarks	Controls should be >95% ok; if >5 % (2/28) plants are undecided or off-type, the experiment should be repeated



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## ANNEX II



### TECHNICAL QUESTIONNAIRE

to be completed in connection with an application for Community Plant Variety Rights  
Please answer all questions. A question without any answer will lead to a non-attribution  
of an application date. In cases where a field / question is not applicable, please state so.

1. **Botanical taxon:** Name of the genus, species or sub-species to which the variety belongs and common name

*Lactuca sativa* L.

LETTUCE

2. **Applicant(s):** Name(s) and address(es), phone and fax number(s), Email address, and where appropriate name and address of the procedural representative

3. **Variety denomination**

a) Where appropriate proposal for a variety denomination:

b) Provisional designation (breeder's reference):

<b>4. Information on origin, maintenance and reproduction of the variety</b>			
<b>4.1 Breeding, maintenance and reproduction of the variety.</b> Please indicate breeding scheme, parents and other relevant information.			
<b>4.2 Geographical origin of the variety:</b> the region and the country in which the variety was bred or discovered and developed			
<b>5. Characteristics of the variety to be indicated</b> (the number in brackets refers to the corresponding characteristic in the CPVO Protocol; please mark the state of expression which best corresponds).			
	<b>Characteristics</b>	<b>Example varieties</b>	<b>Note</b>
<b>5.1 (1)</b>	<b>Seed: colour</b>		
	white	Verpia	1 [ ]
	yellow	Durango	2 [ ]
	black	Kagraner Sommer	3 [ ]
<b>5.2 (16)</b>	<b>Leaf: hue of green colour of outer leaves</b>		
	absent	Donatello, Verpia	1 [ ]
	yellowish	Dorée de printemps	2 [ ]
	greyish	Celtuce, Du bon jardinier	3 [ ]
	reddish	Lollo rossa, Revolution, Rosa	4 [ ]
<b>5.3 (18)</b>	<b>Leaf: anthocyanin coloration</b>		
	absent	Fiorella, Sunrise	1 [ ]
	present	Commodore, Pirat	9 [ ]

Characteristics		Example varieties	Note
<b>5.4</b> <b>(33)</b>	<b>Time of beginning of bolting under long day conditions</b>		
	very early	Blonde à couper améliorée	1 [ ]
	early	Gotte à graine blanche	3 [ ]
	medium	Carelia	5 [ ]
	late	Hilde II	7 [ ]
	very late	Erika, Kinemontepas, Rex	9 [ ]
<b>5.5</b> <b>(37.7)</b>	<b>Resistance to downy mildew (<i>Bremia lactucae</i>), Isolate BI:16</b>		
	absent	Cobham Green, Hilde II	1 [ ]
	present	Argelès, Ninja	9 [ ]
<b>5.6</b>	<b>Growth type at harvest maturity</b>		
	Butterhead lettuce	Clario, Merveille des quatre saisons, Verpia	1 [ ]
	Crisp lettuce	Blonde de Paris (Batavia), Calmar, Saladin (Iceberg)	2 [ ]
	Cos lettuce (Roman lettuce)	Blonde maraîchère (Roman types)	3 [ ]
	“Grasse” or Latin lettuce	Bibb, Sucrine	4 [ ]
	Cutting or Gathering lettuce	Frisée d’Amérique, Lollo rossa, Oakleaf, Salad Bowl	5 [ ]
	Stem lettuce	Celtuce	6 [ ]
<b>6.</b>	<b>Similar varieties and differences from these varieties:</b>		
Denomination of similar variety	Characteristic in which the similar variety is different <sup>1)</sup>	State of expression of similar variety	State of expression of candidate variety
<p><sup>1)</sup> In the case of identical states of expressions of both varieties, please indicate the size of the difference</p>			

**7. Additional information which may help to distinguish the variety**

**7.1 Resistance to pests and diseases**

	absent	present	not tested
i) Resistance to Downy Mildew ( <i>Bremia lactucae</i> )			
a) Isolate BI: 2 (char. 39.1)	[ ]	[ ]	[ ]
b) Isolate BI: 5 (char. 39.2)	[ ]	[ ]	[ ]
c) Isolate BI: 7 (char. 39.3)	[ ]	[ ]	[ ]
d) Isolate BI: 12 (char. 39.4)	[ ]	[ ]	[ ]
e) Isolate BI: 14 (char. 39.5)	[ ]	[ ]	[ ]
f) Isolate BI: 15 (char. 39.6)	[ ]	[ ]	[ ]
g) Isolate BI: 17 (char. 39.8)	[ ]	[ ]	[ ]
h) Isolate BI: 18(char. 39.9)	[ ]	[ ]	[ ]
i) Isolate BI: 20 (char. 39.10)	[ ]	[ ]	[ ]
j) Isolate BI: 21 (char. 39.11)	[ ]	[ ]	[ ]
k) Isolate BI: 22 (char. 39.12)	[ ]	[ ]	[ ]
l) Isolate BI: 23 (char. 39.13)	[ ]	[ ]	[ ]
m) Isolate BI: 24 (char. 39.14)	[ ]	[ ]	[ ]
n) Isolate BI: 25 (char. 39.15)	[ ]	[ ]	[ ]
o) Isolate BI: 26 (char. 39.16)	[ ]	[ ]	[ ]
p) Isolate BI: 27 (char. 39.17)	[ ]	[ ]	[ ]
ii) Resistance to Lettuce Mosaic Virus (LMV)			
Strain LS-1 (char. 40)	[ ]	[ ]	[ ]
iii) Resistance to <i>Nasonovia ribisnigri</i> biotype Nr: 0	[ ]	[ ]	[ ]
iv) Other resistances (specify)	[ ]	[ ]	[ ]

**7.2 Special conditions for the examination of the variety**

YES, please specify

NO

**7.3 Other information**

YES, please specify

NO

**8. GMO-information required**

The variety represents a Genetically Modified Organism within the meaning of Article 2(2) of Council Directive 2001/18/EC of 12/03/2001.

YES

NO

If yes, please add a copy of the written attestation of the responsible authorities stating that a technical examination of the variety under Articles 55 and 56 of the Basic Regulation 2100/94 does not pose risks to the environment according to the norms of the above-mentioned Directive.

**9. Information on plant material to be examined**

**9.1** The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

**9.2** The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

- |   |                              |                             |
|---|------------------------------|-----------------------------|
| (a) Microorganisms (e.g. virus, bacteria, phytoplasma)      | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| (b) Chemical treatment (e.g. growth retardant or pesticide) | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| (c) Tissue culture  | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| (d) Other factors   | <input type="checkbox"/> Yes | <input type="checkbox"/> No |

Please provide details of where you have indicated "Yes":

**10. Possible place of the technical examination**

In case the CPVO needs to arrange a technical examination for this candidate variety, there might be more than one examination office entrusted by the CPVO suitable to grow your variety. In this case, the Office will decide on the place of the technical examination but you might wish to express here a preference in respect of an examination office. The available entrusted examination offices for that species can be found in the S2 Gazette under <http://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette>

I/we hereby declare that to the best of my/our knowledge the information given in this form is complete and correct.

Date

Signature

Name

[End of document]