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European Union
Community Plant Variety Office

PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Valerianella locusta L. and *Valerianella eriocarpa* Desv.

CORNSALAD

UPOV Species Code: VLRNL_LOC; VLRNL_ERI

Adopted on 21/03/2007

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/75/7 dated 05/04/2006 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies for all varieties of ***Valerianella locusta* L.** and ***Valerianella eriocarpa* Desv.**

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) 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 corn salad. 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 factors e.g. presence of pests, 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. The characteristics used for grouping could be the following:

a) Leaf: length (characteristic 3)

b) Leaf: profile of apical part in longitudinal section (characteristic 9)

c) Leaf: intensity of green colour (characteristic 11)

d) Seed: size (characteristic 19)

e) Seed: collar (characteristic 20)

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 40 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.

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ANNEXES TO FOLLOW

ANNEX I

PAGE

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.
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

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.

ANNEX II

Technical Questionnaire

ANNEX I

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

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1.	**1.**	**QN**	**Plant: attitude**		
	(*)	**VG**	erect	Elan	1
			semi-erect	Verte de Louviers	3
			horizontal	Valgros	5
2.	**2.**	**QN**	**Plant: diameter**		
	(*)	**VG**	very small		1
			small	Coquille de Louviers	3
			medium	Verte de Louviers	5
			large	Verte de Cambrai	7
			very large	A grosse graine	9
3.	**3.**	**QN**	**Leaf: length**		
	(*)	**MS/VG**	short	Coquille de Louviers	3
			medium	Verte à coeur plein 2	5
G			long	A grosse graine	7
4.	**4.**	**QN**	**Leaf: width**		
	(*)	**MS/VG**	narrow	Verte d'Etampes	3
			medium	A grosse graine, Verte de Cambrai	5
			broad	Palace, Rodion	7
5.	**5.**	**QN**	**Leaf: ratio length/width**		
		MS/VG	small		3
			medium		5
			large		7

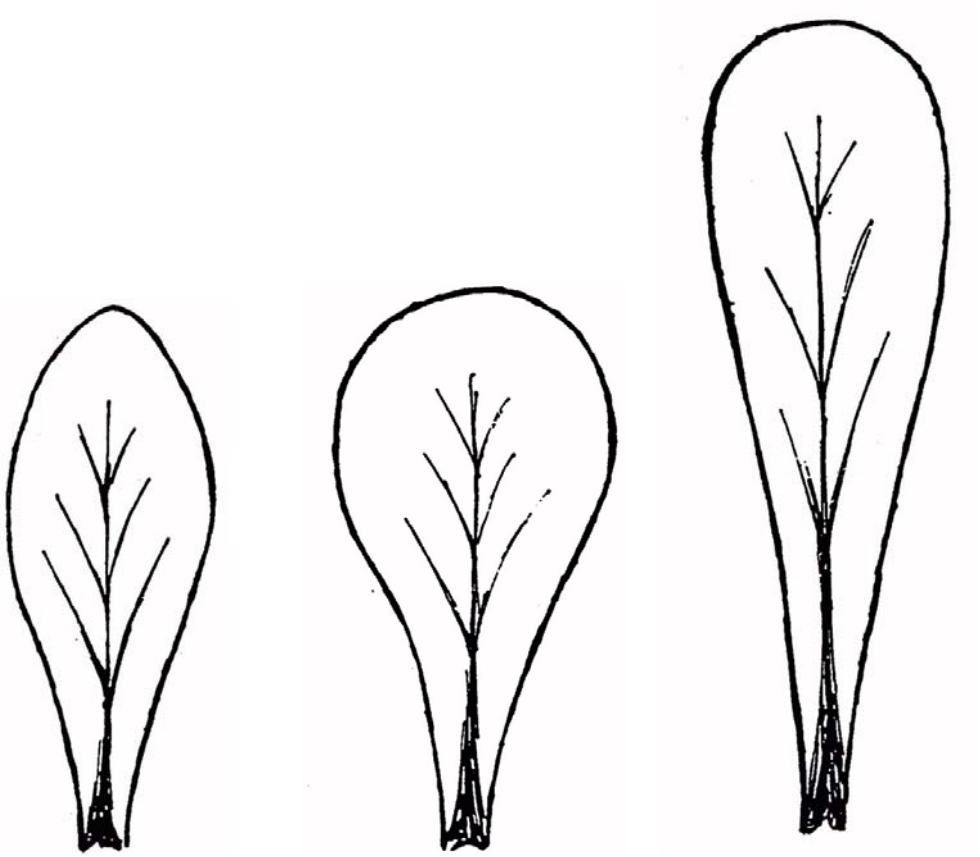
CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6.	**6.**	**PQ**	**Leaf: shape**		
(+)	**(+)**	**VG**	elliptic	Verte de Louviers	1
	(*)		broad spatulate	Verte à coeur plein 2	2
			narrow spatulate	A grosse graine	3
7.	**7.**	**QN**	**Leaf: glossiness**		
		VG	weak	D'Italie à feuille de laitue	3
			medium	Verte maraîchère	5
			strong	Verte de Louviers	7
8.	**8.**	**QN**	**Leaf: profile in cross-section**		
		VG	concave		1
			flat	Coquille de Louviers	2
			convex	Verte à coeur plein 2	3
9. **(+)**	**9.** **(+)**	**QN**	**Leaf: profile of apical part in longitudinal section**		
	(*)	**VG**	concave	Coquille de Louviers	1
			flat	Gala, Verte à cœur plein 2	2
G			convex	Verte d'Etampes	3
10.	**10.**	**QN**	**Leaf: torsion**		
		VG	absent or very weak		1
			weak	Dante	3
			medium	A grosse graine	5
			strong	Topaze	7
11.	**11.**	**QN**	**Leaf: intensity of green colour**		
	(*)	**VG**	light	Verte maraîchère	3
			medium	Verte de Rouen	5
G			dark	Verte à coeur plein 2	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
12.	**12.**	**QL**	**Leaf: dentation (outer leaves)**		
		VG	absent	A grosse graine, Coquille de Louviers	1
			present	Saphir, Sapiana	9
13.	**13.**	**QN**	**Leaf: thickness**		
		VG	thin	Valgros	3
			medium		5
			thick	Verte d'Etampes	7
14.	**14.**	**QN**	**Leaf: prominence of veins**		
	(*)	**VG**	weak	Verte de Louviers	3
			medium	Progres	5
			strong	Toendra, Vit	7
15.	**15.**	**QN**	**Leaf: blistering**		
		VG	absent or very weak	A grosse graine, Baron	1
			weak		3
			medium	D'Italie à feuille de laitue, Saphir	5
			strong	Progres	7
			very strong		9
16.	**16.**	**QN**	**Time of beginning of bolting (10% of plants)**		
		MG	very early	Valgros	1
			early	Verte à coeur plein 2	3
			medium	Verte d'Etampes	5
			late	Baikal	7
17.	**17.**	**QL**	**Flower stem: fasciation**		
		VG	absent	A grosse graine, Coquille de Louviers	1
			present	Jobra, Jovis	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
18.	**18.**	**QN**	**Flower stem: anthocyanin coloration**		
		VG	weak	A grosse graine	3
			medium	Valvert	5
			strong	Pustade	7
19.	**19.**	**QN**	**Seed: size**		
(+)		**VG**	small	D'Italie à feuille de laitue, Deutscher	3
	(*)		medium	Vit	5
G			large	A grosse graine	7
20.	**20.**	**QL**	**Seed: collar**		
(+)	**(+)**	**VG**	absent	Deutscher	1
G	**(*)**		present	D'Italie à feuille de laitue	9
21. **(+)**	**21.** **(+)**	**QL** **VG**	**Resistance to downy mildew (*Peronospora valerianella)***		
21.1	**21.1**		**Strain 1**		
			absent		1
			present		9
21.2	**21.2**		**Strain 2**		
			absent		1
			present		9

EXPLANATIONS AND METHODS

Ad. 6: Leaf: shape



1
elliptic

2
broad spatulate

3
narrow spatulate

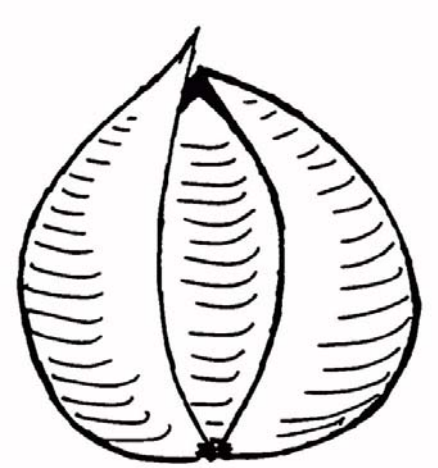
Ad. 9: Leaf: profile of apical part in longitudinal section

1
concave

2
flat

3
convex

Ad. 20: Seed: collar



1
absent

9
present

Ad. 21: Resistance to downy mildew (*Peronospora valerianella*)

Strain(s) used:	Races 1, 2
Maintenance of strains:	
Nature of medium:	Conservation of strains in the form of oospores associated with naturally contaminated seeds.
Particular conditions:	Storage of batches of contaminated seeds in waterproof sachets at 4°C, on different varieties.
Comments:	Since mildew spores are fragile, medium-term conservation (a few months) on contaminated leaves kept in a freezer (-20°C) is particularly delicate.
Production of inoculum:	At the beginning of the test period, inoculum is produced from naturally contaminated seeds (sanitary analysis). Broadcast sowing, about 200-250 seeds per box. Germination and development of plant germs in a cold tunnel, 10 to 15°C. From the time of appearance of the first leaf, the boxes are placed in a plastic cage or are covered with a plastic lid (mini-glasshouse), in order to generate moisture on the plants.

10 to 12 days after sowing, the first symptoms appear on the plants produced from the infected seeds. The cotyledons and leaves have a rolled aspect. The sick leaves are recovered in order to multiply the inoculum or for an infection. The spores are collected on a recent sporulation (night time). They are suspended in a small amount of permuted water, with 20 tween added (1 drop per 100 ml) and filtered on stamens. The concentration in spores is adjusted to 10^5 spores/ml. The inoculum is kept on a bed of ice.

Sowing: Sowing in plugs (5 x 5 cm), at a rate of 2 to 3 seeds per plug, in order to conserve only one seed per plug.

Conduct of the test:

Plant stage: First leaf stage

Number of plants studied: 40 plants per variety and 10 plants of a control variety.

Cultivation conditions: 10 to 15ºC before inoculation / 8 to 15ºC after inoculation: the difference in temperature is important.

Implantation: Cold glasshouse prior to inoculation / cold tunnel (anti-freeze) after inoculation.

Inoculation: Spraying of a suspension of spores at 10^5 spores/ml, using an ECOSPRAY type sprayer, then the plants are covered for 48 hours in a plastic cage.

Duration of the test: Sowing-inoculation: approximately 10-12 days
Inoculation-reading: 12-15 days.

Test reading: Beginning of sporulation on sensitive plants approximately 12 days after the inoculation.

Test reliability:

Differential hosts to be used:

Hosts	Pathotype 1 (Verte de Cambrai)	Pathotype 2 (Gala)
Verte de Cambrai	S	R
Verella	R	S
Gala	R	S

S = Sensitive, R = Resistant

LITERATURE

Fascicule du CTPS - Novembre 1995: Tests de résistance aux maladies, Plantes potagères.

ANNEX II

European Union
Community Plant Variety Office

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

***Valerianella locusta* L. and *Valerianella eriocarpa* Desv.**

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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):

4. Information on origin, maintenance and reproduction of the variety

4.1 Breeding, maintenance and reproduction of the variety.
Please indicate breeding scheme, parents and other relevant information.

4.2 Geographical origin of the variety: the region and the country in which the variety was bred or discovered and developed

5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in the CPVO Protocol; please mark the state of expression which best corresponds).

Characteristics		Example varieties	Note
5.1 (3)	**Leaf: length**		
	short	Coquille de Louviers	3 [ ]
	medium	Verte à coeur plein 2	5 [ ]
	long	A grosse graine	7 [ ]
5.2 (9)	**Leaf: profile of apical part in longitudinal section**		
	concave	Coquille de Louviers	1 [ ]
	flat	Gala, Verte à coeur plein 2	2 [ ]
	convex	Verte d'Etampes	3 [ ]
5.3 (11)	**Leaf: intensity of green colour**		
	light	Verte maraïchère	3 [ ]
	medium	Verte de Rouen	5 [ ]
	dark	Verte à coeur plein 2	7 [ ]

Characteristics		Example varieties	Note
5.4 (19)	**Seed: size**		
	small	D'Italie à feuille de laitue, Deutscher	3 [ ]
	medium	Vit	5 [ ]
	large	A grosse graine	7 [ ]
5.4 (20)	**Seed: collar**		
	absent	Deutscher	1 [ ]
	present	D'Italie à feuille de laitue	9 [ ]

6. Similar varieties and differences from these varieties:

Denomination of similar variety	Characteristic in which the similar variety is different[1)]	State of expression of similar variety	State of expression of candidate variety

1) In the case of identical states of expressions of both varieties, please indicate the size of the difference

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 (*Peronospora valerianella*)			
a) Strain 1 (char. 21.1)	[ ]	[ ]	[ ]
b) Strain 2 (char. 21.2)	[ ]	[ ]	[ ]
ii) 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)	[ ] Yes	[ ] No
(b) Chemical treatment (e.g. growth retardant or pesticide)	[ ] Yes	[ ] No
(c) Tissue culture	[ ] Yes	[ ] No
(d) Other factors	[ ] Yes	[ ] No

Please provide details of where you have indicated “Yes”:

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]