



PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

***Malus* Mill.**

APPLE ROOTSTOCKS

UPOV Code: MALUS

Adopted on 11/03/2010

Entered into force on 12/03/2010

I SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the Council Regulation 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/163/3 dated 24/03/1999 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies for all vegetatively propagated rootstock varieties of *Malus Mill.* If characteristics of the flower, the fruit or the seed are necessary to establish distinctness, the CPVO protocol for apple fruit varieties (*Malus domestica*, TP14/2) should be used for those characteristics, if applicable.

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 final dates for request for technical examination and sending of Technical Questionnaire by the CPVO as well as submission date, quantity and quality of plant material by the applicant can be found in the CPVO website (www.cpvo.europa.eu).

Quality of plants: Should not be less than the standards laid down in Council Directive 2000/29/EC and its amendments concerning quarantine organisms, and Council Directives 92/34/EEC and 93/48/EEC and their amendments concerning organisms impairing quality, at the date of adoption of this protocol; please refer to "Eur-Lex" for the full text and in case of any subsequent amendments to the two aforesaid Directives.

Healthy plant material of the candidate variety should be delivered to the test station in accordance with the requirements outlined in the instructions sent by the CPVO for the submission of plant material, and which can also be consulted in the relevant entries for apple rootstocks within the S2 Gazette and the CPVO website. In particular with respect to the phytosanitary requirements, the plant material must be accompanied by a valid certificate from a recognised authority attesting to the fact that the plant material sent for the DUS technical examination has shown negative laboratory test results for the list of pests and pathogens outlined in the pertinent entry of the examination office in the S2 Gazette/CPVO website, where the candidate apple rootstock variety is to undergo its DUS technical examination.

Chemical 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.

Labelling of individual..... - Species
plants in sample: - 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 apple rootstocks. 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. The characters used for grouping could be the following:

- a) Plant: habit of shoot (characteristic 3)
- b) Plant: growth of shoot (characteristic 4)
- c) Expanding leaf: anthocyanin coloration of blade (characteristic 18)
- d) Time of beginning of bud burst (characteristic 32)

5. Trial designs and growing conditions

The minimum duration of tests (independent growing cycles) will normally include at least two similar growing periods. 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 15 plants. From the 15 plants, 10 of these plants should be cut back annually in the stoolbed to enable the observation of all the characteristics in this protocol TP-163/1. The other 5 plants should be grown to produce trees, in case characteristics of the adult tree are needed for the establishment of distinctness utilising the current version of the CPVO apple fruit protocol TP-14.

Unless otherwise indicated, all observations should be made on 10 plants or parts taken from each of 10 plants in the stoolbed.

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 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
6-25	1

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 vegetation periods but in some cases three vegetation 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 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 **12/03/2010**. Any ongoing DUS examination of candidate varieties with observations 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 at the time the first observations are made on characteristics in an independent growing cycle.

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.

ANNEXES TO FOLLOW

ANNEX I

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Legend:

- (+) See Explanations on the Table of characteristics
- (a)-(c) See Explanations on the Table of Characteristics
- G Grouping 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.

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ANNEX II

Technical Questionnaire

ANNEX I

TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTIONS

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
1.	1.	VG	Plant: vigour		
QN	(*)	(b)	weak	M 27	3
			medium	M 7, M 26	5
			strong	MM 106	7
2.	2.	VG	Plant: number of shoots		
QN		(b)	very few	M 27	1
			few	M 9	3
			medium	M 26	5
			many	MM 106, MM 111	7
			very many	M 25	9
3.	3.	VG	Plant: habit of shoot		
QN	(*)	(b)	upright	M 4	1
			spreading	M9	2
G			drooping	Marubakaido	3
4.	4.	VG	Plant: growth of shoot		
QL	(*)	(b)	straight	M 9	1
G			wavy or zigzag	M 2, M 25	2
5.	5.	VG	Shoot: pubescence (on upper half of shoot)		
QN	(*)	(b)	absent or very weak		1
			weak	B 9, M 26	3
			medium	M 27	5
			strong	M 9	7
			very strong	Crab C	9

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
6.	6.	VG	Shoot: glossiness of bark		
QN	(*)	(b)	absent or very weak		1
			weak	M 26	3
			medium		5
			strong	M 27	7
			very strong		9
7.	7.	VG	Shoot: thickness (at midlength)		
QN	(*)	(b)	thin	M 7, M 27	3
			medium	MM 111	5
			thick	MM 106	7
8.	8.	VG	Shoot: length of internodes (at midlength)		
QN	(*)	(b)	short	M 25	3
			medium	M 26	5
			long	M 7	7
9.	9.	VG	Shoot: number of lenticels		
QN	(*)	(b)	absent or very few		1
			few	M 9	3
			medium	M 26	5
			many	M 2, MM 111	7
			very many	MM 104	9
10.	10.	VG	Shoot: size of lenticels		
QN		(b)	small		3
			medium	M 9, M 26	5
			large	M 2	7
11.	11.	VG	Shoot: shape of lenticels		
PQ		(b)	elliptic	M 25	1
			broad elliptic	M 26, MM 111	2
			circular	M 9, M 27	3

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
12.	12.	VG	Shoot: predominant colour on sunny side		
PQ	(*)	(b)	greenish brown		1
			reddish brown	M 9	2
			medium brown	M 25, M 27	3
			dark brown	B 9, M 2, M 26, MM 106	4
13.	13.	VG	Shoot: size of bud		
QN	(*)	(b)	small	M 25, MM 111	3
			medium	MM 106	5
			large	M2, M 9, M 27	7
14.	14.	VG	Shoot: shape of tip of bud		
(+)	(+)	(b)	pointed	M 9, M 27	1
QL			rounded	Bemali, MM 111	2
15.	15.	VG	Shoot: position of bud relative to axis		
(+)	(+)	(b)	adpressed	MM 106	1
QN			slightly held out	M 9, M 26	2
			markedly held out		3
16.	16.	VG	Shoot: size of bud support		
(+)	(+)	(b)	small	M 9	3
QN			medium	M 7, M 27	5
			large	M 2	7
17.	17.	VG	Shoot: colour of growing tip		
PQ	(*)	(b)	whitish	M 25	1
			greenish	M 2, M 27, MM 106	2
			reddish	M 9	3
			dark purple	B 9, M 10, M 26	4

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
18.	18.	VG	Expanding leaf: anthocyanin coloration of blade		
QL	(*)		absent	M 27	1
G			present	B 9	9
19.	19.	VG	Expanding leaf: hue of anthocyanin coloration of blade		
QL	(*)		bronze	P 22	1
			purple	B 9	2
20.	20.	VG	Leaf blade: attitude in relation to shoot		
(+)	(+)	(c)	semi-upwards	MM 111	3
QN			outwards	M 7, MM 106	5
			semi-downwards		7
21.	21.	MG/VG	Leaf blade: length		
QN	(*)	(c)	short	M 26, M 27	3
			medium	MM 111	5
			long	M 9, P 16	7
22.	22.	MG/VG	Leaf blade: width		
QN	(*)	(c)	narrow	M 26	3
			medium	M 9, M 27	5
			broad	P 14	7
23.	23.	VG/MG	Leaf blade: ratio length/width		
QN	(*)	(c)	small	M 7	3
			medium	M 26	5
			large	P 16	7
24.	24.	VG	Leaf blade: profile in cross section		
PQ	(*)	(c)	concave	M 27, M 111	1
			straight	M 9	2
			convex	M 25	3

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
25.	25.	VG	Leaf blade: length of pointed tip		
QN	(*)	(c)	short	M 27	3
			medium	M 9	5
			long	P 16	7
26.	26.	VG	Leaf blade: incisions of margin		
QL	(*)	(c)	crenate	M 9	1
			serrate	M 26, M 27	2
27.	27.	VG	Leaf blade: pubescence on lower side		
QN		(c)	weak	M 9	3
			medium	M 27	5
			strong	MM 106	7
28.	28.	VG	Leaf blade: anthocyanin coloration of veins		
QN	(*)	(c)	weak	M 9	1
			medium	M 26	3
			strong	MM 106, MM 109	5
29.	29.	VG	Petiole: length		
QN	(*)	(c)	short	M 26, M 27	3
			medium	M 9	5
			long	MM 106, MM 111	7
30.	30.	MG/VG	Leaf: ratio length of blade/length of petiole		
QN	(*)	(c)	small		3
			medium	B 9, M 9	5
			large	P 2, P 16	7
31.	31.	VG	Stipule: size		
QN	(*)		small	M 27	3
			medium	M 9, M 26	5
			large	MM 106	7

CPVO N°	UPOV N°	Stage, method	Characteristics	Examples	Note
32.	32.	MG	Time of beginning of shoot growth		
QN	(*)		very early	P 16	1
(+)			early	M 9, MM 106	3
			medium	M 25	5
			late	MM 111	7
G			very late	M 26	9

EXPLANATIONS AND METHODS

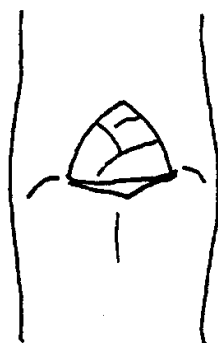
1 *Explanations covering several characteristics*

Characteristics containing the following key in the third column of the Table of Characteristics should be examined as indicated below:

- (a) Observations of vegetative characteristics should be made on plants which are cut back annually in the stoolbed.
- (b) Observations on the plant and the shoot should be made in the middle third of the one-year-old shoot in the dormant season.
- (c) Observations on the leaf should be made on fully developed leaves from the middle third of a vigorous shoot in summer.

2 *Explanations covering several characteristics*

Ad. 14: Shoot: shape of tip of bud

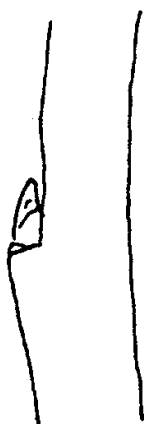


1
pointed



2
rounded

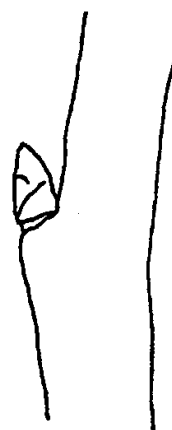
Ad. 15: Shoot: position of bud relative to axis



1
adpressed



2
slightly held out



3
markedly held out

Ad. 16: Shoot: size of bud support



3
small

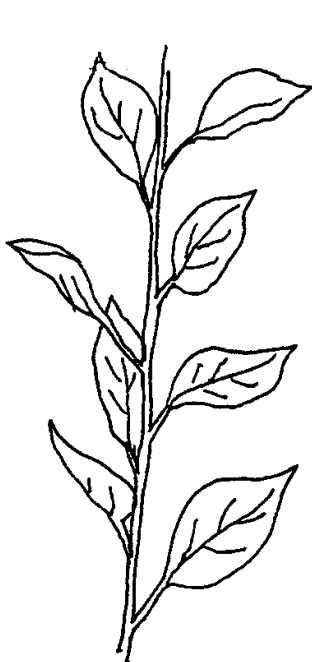


5
medium

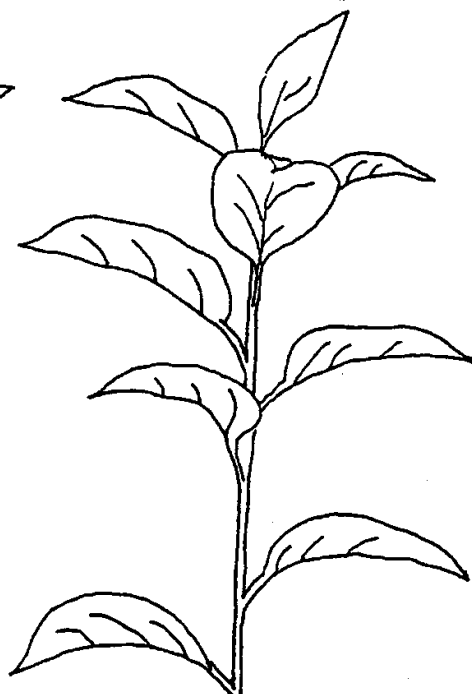


7
large

Ad. 20: Leaf blade: attitude in relation to shoot



3
semi-upwards



5
outwards



7
semi-downwards

Ad. 32: Time of beginning of shoot growth

The time of beginning of shoot growth should be considered as the time at the beginning of the vegetation period, when the first young shoots break through the soil surface.

LITERATURE

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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

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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 Origin

- (a) Seedling of unknown parentage []
- (b) Produced by controlled pollination (indicate parent variety) []
- Seed bearing parent (indicate parent)
 - Pollen parent (indicate parent)
- (c) Produced by open pollination of
(indicate seed bearing parent plant) []
- (d) Mutation or sport from (indicate original parent variety) []
- (e) Discovery (indicate where and when) []

4.2 *In vitro* propagation

The plant material of the candidate variety has been obtained by *in vitro* propagation

[] YES

[] NO

4.3 Virus status

- (a) The variety is free from all known viruses as follows
(indicate from which viruses).....[]
- (b) The plant material is virus tested
(indicate against which viruses).....[]
- (c) The virus status is unknown[]

4.4 Other information on genetic origin and breeding method:

4.5 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)	Plant: habit of shoot		
	upright	M 4	3 []
	spreading	M9	5 []
	drooping	Marubakaido	7 []
5.2 (4)	Plant: growth of shoot		
	straight	M 9	1 []
	wavy or zigzag	M 2, M 25	2 []
5.3 (18)	Expanding leaf: anthocyanin coloration of blade		
	absent	M 27	1 []
	present	B 9	9 []

Characteristics		Example varieties	Note
5.4	Time of beginning of shoot growth		
(32)			
	very early	P 16	1 []
	early	M 9, MM 106	3 []
	medium	M 25	5 []
	late	MM 111	7 []
	very late	M 26	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			
7.2 Special conditions for the examination of the variety			
☐ YES, please specify			
☐ NO			

7.3 Other information

A representative colour photograph of the variety should accompany the Technical Questionnaire.

☐ 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]