



**CPVO**

Community Plant Variety Office

## DISCLAIMER

The present version of the national guideline has been accepted by the President of the CPVO for its use in technical examinations carried out on behalf of the CPVO or for the take-over of reports serving as a basis for a CPVO decision.

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

***Trifolium resupinatum L.***

**Persian clover**

UPOV Code: TRFOL\_RES

**Adopted on 01/09/2016**

**Entry into force on 01/09/2016**

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## **1. SUBJECT OF THE PROTOCOL AND REPORTING**

### **1.1 Scope of the technical protocol**

This Technical Protocol applies to all varieties of *Trifolium pratense* L.

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures are based on documents agreed by the International Union for the Protection of New Varieties of Plants (UPOV), such as the General Introduction to DUS (UPOV Document TG/1/3 [http://www.upov.int/en/publications/intro\\_dus.htm](http://www.upov.int/en/publications/intro_dus.htm)), its associated TGP documents (<http://www.upov.int/en/publications/tgp/>).

### **1.2 Entry into Force**

The present protocol enters into force on **01.09.2016**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. 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 submitting of plant material for the first test period.

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

### **1.3 Reporting between Examination Office and CPVO and Liaison with Applicant**

#### 1.3.1 Reporting between Examination Office and CPVO

The Examination Office shall deliver to the CPVO a preliminary report ("the preliminary report") no later than two weeks after the date of the request for technical examination by the CPVO.

The Examination Office shall also deliver to the CPVO a report relating to each growing period ("the interim report") and, when the Examination Office considers the results of the technical examination to be adequate to evaluate the variety or the CPVO so requests, a report relating to the examination ("the final report").

The final report shall state the opinion of the Examination Office on the distinctness, uniformity and stability of the variety. Where it considers those criteria to be satisfied, or where the CPVO so requests, a description of the variety shall be added to the report. If a report is negative the Examination Office shall set out the detailed reasons for its findings.

The interim and the final reports shall be delivered to the CPVO as soon as possible and no later than on the deadlines as laid down in the designation agreement.

#### 1.3.2 Informing on problems in the DUS test

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 permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

#### 1.3.3 Sample keeping in case of problems

If the technical examination has resulted in a negative report, the CPVO shall inform the Examination Office as soon as possible in case that a representative sample of any relevant testing material shall be kept.

## **2. MATERIAL REQUIRED**

### **2.1 Plant material requirements**

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on <http://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette> in the special issue S2 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

## **2.2 Informing the applicant of plant material requirements**

The CPVO informs the applicant that

- he is responsible for ensuring compliance with any customs and plant health requirements.
- the plant material supplied should be visibly healthy, not lacking in vigour, nor affected by any important pest or disease.
- 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 it has been treated, full details of the treatment must be given.

## **2.3 Informing about problems on the submission of material**

The Examination Office shall report to the CPVO immediately in cases where the test material of the candidate variety has not arrived in time or in cases where the material submitted does not fulfil the conditions laid down in the request for material issued by the CPVO.

In cases where the examination office encounters difficulties to obtain plant material of reference varieties the CPVO should be informed.

## **3. METHOD OF EXAMINATION**

### **3.1 Number of growing cycles**

The minimum duration of tests should normally be two independent growing cycles.

### **3.2 Testing Place**

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness"

[http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp\\_9\\_1.pdf](http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_9_1.pdf).

### **3.3 Conditions for Conducting the Examination**

The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

#### **(a) Stage of development for the assessment**

"The optimum stage of development for the assessment of each characteristic is indicated by a number in the third column of the Table of Characteristics. The stages of development denoted by each number are described in Chapter 8 [...]."

#### **(b) Type of plot for observation**

The recommended type of plot in which to observe the characteristic is indicated by the following key in the second column of the Table of Characteristics:

- |    |               |
|----|---------------|
| A: | spaced plants |
| B: | row plot      |
| C: | special test  |

### **3.4 Test design**

3.4.1 Each test should be designed to result in a total of at least 60 spaced plants and 5 meters of row plot."

3.4.2 The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle."

### **3.5 Additional tests**

In accordance with Article 83(3) of Council Regulation 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, an additional test may be undertaken providing that a technically acceptable test procedure can be devised.

Additional 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.

### **3.6 Constitution and maintenance of a variety collection**

The process for the constitution and the maintenance of a variety collection can be summarized as follows:

Step 1: Making an inventory of the varieties of common knowledge

Step 2: Establishing a collection ("variety collection") of varieties of common knowledge which are relevant for the examination of distinctness of candidate varieties

Step 3: Selecting the varieties from the variety collection which need to be included in the growing trial or other tests for the examination of distinctness of a particular candidate variety.

3.6.1 The variety collection shall comprise variety descriptions and living plant material, thus a living reference collection. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database

3.6.2 The EO shall collect and maintain living plant material of varieties of the species concerned in the variety collection.

3.6.3 The living variety collection shall cover at least those varieties that are suitable to climatic conditions of a respective EO.

3.6.4 The inventory shall include varieties protected under National PBR (UPOV contracting parties) and Community PBR, varieties registered in the Common Catalogue, the OECD list, the Conservation variety list and varieties in trade or in commercial registers for those species not covered by a National or the Common Catalogue.

3.6.5 The EO shall maintain seeds in conditions which will ensure germination and viability, periodical checks, and renewal as required. For the renewal of existing living material the identity of replacement living plant material shall be verified by conducting side-by-side plot comparisons between the material in the collection and the new material.

## **4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY**

The prescribed procedure is to assess distinctness, uniformity and stability in a growing trial.

### **4.1 Distinctness**

#### **4.1.1 General recommendations**

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 9 'Examining Distinctness' ([http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp\\_9\\_1.pdf](http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_9_1.pdf)) prior to making decisions regarding distinctness.

Further guidance is provided in documents TGP/9 "Examining Distinctness" and TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability".

#### **4.1.2. Consistent differences**

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

#### **4.1.3 Clear differences**

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Technical Protocols are familiar with the recommendations contained in the UPOV-General Introduction to DUS prior to making decisions regarding distinctness.

"4.1.3.2 "If distinctness is assessed by the combined over years distinctness analysis (COYD) the difference between two varieties is clear if the respective characteristics are different at the 1% significance level or less ( $p < 0.01$ ) in a test over either two or three years."

#### 4.1.4 Number of plants/parts of plants to be examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 60 plants or parts taken from each of 60 plants and any other observations made on all plants in the test, disregarding any off-type plants. In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 1.

#### 4.1.5 Method of observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the third column of the Table of Characteristics (see document TGP/9 "Examining Distinctness", Section 4 "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

Type of observation: visual (V) or measurement (M)

"Visual" observation (V) is an observation made on the basis of the expert's judgment. For the purposes of this document, "visual" observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. color charts). Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

Type of record: for a group of plants (G) or for single, individual plants (S)

For the purposes of distinctness, observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded as records for a number of single, individual plants or parts of plants (S). In most cases, "G" provides a single record per variety and it is not possible or necessary to apply statistical methods in a plant-by-plant analysis for the assessment of distinctness."

In cases where more than one method of observing the characteristic is indicated in the Table of Characteristics (e.g. VG/MG), guidance on selecting an appropriate method is provided in document TGP/9, Section 4.2.

## 4.2 **Uniformity**

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 10 'Examining Uniformity' ([http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp\\_10\\_1.pdf](http://www.upov.int/export/sites/upov/en/publications/tgp/documents/tgp_10_1.pdf)) prior to making decisions regarding uniformity.

If uniformity is assessed by the combined over years uniformity method (COYU) the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the 1% significance level or less ( $p < 0.01$ ) in a test over two consecutive cycles.

If uniformity is assessed by the combined over years uniformity method (COYU) the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the 0.1% significance level or less ( $p < 0.001$ ) in a test over three consecutive cycles.

## 4.3 **Stability**

4.3.1 It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 11 'Examining Stability'

In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

## 5. GROUPING OF VARIETIES AND ORGANIZATION OF THE GROWING TRIAL

- 5.1** The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.
- 5.2** Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.
- 5.3** The following have been agreed as useful grouping characteristics.
- a) Time of flowering (characteristic 4)
  - b) Stem: length (characteristic 5)
- 5.4** If other characteristics than those from the TP are used for the selection of varieties to be included into the growing trial, the EO shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.

## 6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

### 6.1 Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. 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 or by specific legislation on plant health. In such case, the CPVO should be informed.

#### States of expression and corresponding notes

In the case of qualitative and pseudo-qualitative characteristics, all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines may be abbreviated as follows:

State	Note
small	3
medium	5
large	7

However, it should be noted that all of the following 9 states of expression exist to describe varieties and should be used as appropriate:

State	Note
very small	1
very small to small	2
small	3
small to medium	4
medium	5
medium to large	6
large	7
large to very large	8
very large	9



## 6.2 Example Varieties

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

## 6.3 Legend

G	Grouping characteristic
MG, MS, VG, VS	– see Chapter 4.1.5
QL	Qualitative characteristic
QN	Quantitative characteristic

## 7. TABLE OF CHARACTERISTICS

National N°	Stage, Method	Characteristics	Examples	Note
1.	21-23 QN C/MS	<b>Cotyledon: length</b>		
		short	Stranthwood	3
		medium		5
		long	Laser	7
2.	21-23 QN C/MS	<b>Cotyledon: width</b>		
		narrow		3
		medium		5
		broad	Laser	7
3.	27-29 QN A/VS	<b>Plant: growth habit in time of flowering</b>		
		erect		1
		semi-erect		3
		intermediate	Laser II	5
		semi-prostrate	Nitro Plus	7
		prostrate		9
4.	30-32 QN A/MS  G	<b>Time of flowering</b>		
		very early		1
		early	Lightning	3
		medium	Laser II	5
		late		7
		very late		9
		5.	34-36 QN A/MS  G	<b>Stem: length</b>
very short				1
short	Nitro Plus			3
medium				5
long				7
very long				9

<b>National N°</b>	<b>Stage, Method</b>	<b>Characteristics</b>	<b>Examples</b>	<b>Note</b>	
<b>6.</b>	<b>34-36</b> <b>QN</b> <b>A/MS</b>	<b>Stem: thickness</b>			
		very thin	Stranthwood	1	
		thin	Lightning	3	
		medium	Pasat	5	
		thick		7	
		very thick		9	
<b>7.</b>	<b>34-36</b> <b>QN</b> <b>A/MS</b>	<b>Stem: number of internodes</b>			
		very low		1	
		low		3	
		medium		5	
		high	Laser II	7	
		very high	Pasat	9	
<b>8.</b>	<b>34-36</b> <b>QN</b> <b>A/MS</b>	<b>Leaf: length of medial leaflet</b>			
		short		3	
		medium	Laser	5	
		long		7	
<b>9.</b>	<b>34-36</b> <b>QN</b> <b>A/MS</b>	<b>Leaf: width of medial leaflet</b>			
		narrow		3	
		medium	Lightning	5	
		broad		7	

**8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS**

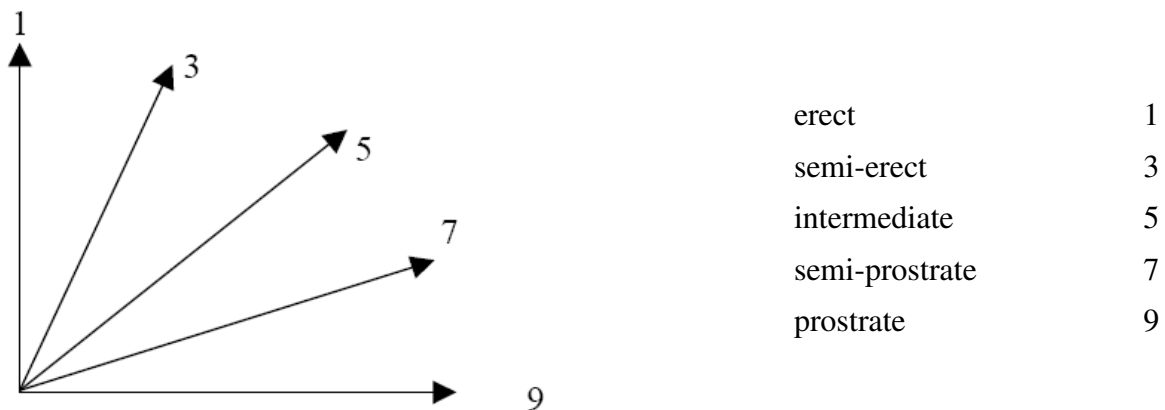
Ad. 1 and 2: Cotyledon: length (1), width (2)

The observation should be made 12-14 days after sowing in greenhouse, when the first leaf is fully developed. If the two cotyledons differ in size, the biggest one should be measured.



Ad. 3: Plant: growth habit in time of flowering

A visual estimate is taken of the angle that the outer shoots make with the horizontal.



Ad. 4: Time of flowering

The observation should be made when 3 heads per plant are flowering.

Ad. 5, 6 and 7: Stem: length (5), thickness (6), number of internodes (7)

The longest stem should be observed including the head within 1-2 weeks after mean date of flowering. The thickness should be measured 2 to 4 cm above tillering node.

Ad. 8, 9 : Leaf: length of medial leaflet and Leaf: width of medial leaflet

All measurements on the leaf should be made within 1 to 2 weeks after the mean date of flowering on the third leaf of the main stem from the top.

## 9. TECHNICAL QUESTIONNAIRE



**CPVO · OCVV**

Community Plant Variety Office  
Office Communautaire des Variétés Végétales

### 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

*Trifolium resupinatum L.*

Persian Clover

**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 the breeding scheme and propagation of the variety</b>			
<b>4.1 Breeding scheme</b> Please indicate breeding scheme, parents, other relevant information			
<b>4.2 Method of propagating the variety</b>			
<p style="margin-left: 20px;">"4.2.1 Seed-propagated varieties</p> <p style="margin-left: 40px;">(a) Self-pollination ..... [ ]</p> <p style="margin-left: 40px;">(b) Cross-pollination</p> <p style="margin-left: 80px;">(i) population ..... [ ]</p> <p style="margin-left: 80px;">(ii) synthetic variety..... [ ]</p> <p style="margin-left: 40px;">(c) Hybrid ..... [ ]</p> <p style="margin-left: 40px;">(d) Other ..... [ ]</p> <p style="margin-left: 40px;">(please provide details)"</p>			
<b>5. Characteristics of the variety to be indicated</b> (the number in brackets refers to the corresponding characteristic in the National Protocol; please mark the state of expression which best corresponds).			
	Characteristics	Example varieties	Note
<b>5.1 (4)</b>	<b>Time of flowering</b>		
	very early		1 [ ]
	early	Lightning	3 [ ]
	medium	Laser II	5 [ ]
	late		7 [ ]
	very late		9 [ ]
<b>5.2 (5)</b>	<b>Stem: length</b>		
	very short		1 [ ]
	short	Nitro Plus	3 [ ]
	medium		5 [ ]
	long		7 [ ]
	very long		9 [ ]

<p><b>6. Similar varieties and differences from these varieties:</b>  <i>Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.</i></p>			
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><b>Comments:</b></p>			
<p><sup>1)</sup> In the case of identical states of expressions of both varieties, please indicate the size of the difference</p>			
<p><b>7. Additional information which may help in the examination of the variety</b></p>			
<p><b>7.1 Resistance to pests and diseases</b></p> <p><input type="checkbox"/> YES, please specify</p> <p><input type="checkbox"/> NO</p>			
<p><b>7.2 In addition to the information provided in sections 5 and 6, are there any other characteristics which may help to distinguish the variety?</b></p> <p><input type="checkbox"/> YES, please specify</p> <p><input type="checkbox"/> NO</p>			
<p><b>7.3 Are there any special conditions for growing the variety or conducting the examination?</b></p> <p><input type="checkbox"/> YES, please specify</p> <p><input type="checkbox"/> NO</p>			

**7.4 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 EC/2001/18 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 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":

**9.3** Has the plant material to be examined been tested for the presence of virus or other pathogens?

Yes, (please provide details as specified by the Authority)

No



**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 S3 Gazette under

<http://www.cpvo.europa.eu/main/en/home/technical-examinations/list-of-entrusted-examination-offices-s3>

You will also find in the S2 Gazette further information about submission of plant material and deadlines for numerous major species.

<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]