CPVO · OCVV
Community Plant Variety Office
Office Communautaire des Variétés Végétales

PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Pisum sativum L.

PEA

UPOV Code: PISUM_SAT

Adopted on 06/03/2020

Entry into force on 01/01/2020

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CPVO-TP/007/2 Rev. 3-Corr.

1. SUBJECT OF THE PROTOCOL AND REPORTING

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of ***Pisum sativum* 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 have been agreed by the Administrative Council and 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/export/sites/upov/resource/en/tg_1_3.pdf), its associated TGP documents (http://www.upov.int/tgp/en/) and the relevant UPOV Test Guideline TG/7/10 dated 09/04/2014 (https://www.upov.int/edocs/tgdocs/en/tg007.pdf) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **01.01.2020**. 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 CPVO 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://cpvo.europa.eu/applications-and-examinations/technical-examinations/submission-of-plant-material-s2-publication 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.

The two independent growing cycles should be in the form of two separate plantings.

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/edocs/tgpdocs/en/tgp_9.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.

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 8.3.

3.4 Test design

3.4.1 Each test should be designed to result in a total of at least 100 plants, which should be divided between at least two replicates.

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 Special tests for additional characteritics

In accordance with Article 23 of Implementing Rules N° 874/2009 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.

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 Forms of variety collection

Seed propagated agricultural and vegetable species and fruit species specified on the annex 1 of the entrustment requirements

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 Living Plant Material

Seed propagated agricultural and vegetable species and fruit species specified on the annex 1 of the entrustment requirements

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

3.6.3 Range of the variety collection

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

3.6.4 Making an inventory of varieties of common knowledge for inclusion in the variety collection

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 Maintenance and renewal/update of a living variety collection

Seed propagated species

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/edocs/tgpdocs/en/tgp_9.pdf) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

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.

Decision standards

If distinctness is assessed using the 2 x 1% criterion, the varieties need to be significantly different in the same direction at the 1% level in at least two out of three years in one or more measured characteristics. The tests in each year are based on Student's two-tailed t-test of the differences between variety means with standard errors estimated using the residual mean square from the analysis of the variety x replicate plot means.

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.

If the significance level or statistical methods proposed are not appropriate the method used should be clearly described.

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 20 plants or parts taken from each of 20 plants and any other observations made on all plants in the test, disregarding any off-type plants.

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/edocs/tgpdocs/en/tgp_10.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

This Technical Protocol has been developed for the examination of self-pollinated varieties.

If uniformity is assessed on the basis of off-types, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 100 plants, 3 off-types are allowed.

4.3 Stability

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' (http://www.upov.int/edocs/tgpdocs/en/tgp_11.pd)

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 ORGANISATION 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 organise the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics.

a) Plant: anthocyanin coloration (characteristic 1)

b) Stem: fasciation (characteristic 3)

c) Stem: length (characteristic 4)

d) Stem: number of nodes up to and including first fertile node (characteristic 5)

e) Leaf: leaflets (characteristic 8)

f) Stipule: flecking (characteristic 19)

g) Only varieties with stem fasciation absent: Plant: maximum number of flowers per node (characteristic 24)

h) Pod: length (characteristic 35)

i) Pod: parchment (characteristic 37)

j) Excluding varieties with pod parchment: entire: Pod: thickened wall (characteristic 38)

k) Only varieties with Pod: thickened wall: absent: Pod: shape of distal part (characteristic 39)

l) Pod: curvature (characteristic 40)

m) Pod: colour (characteristic 41)

n) Immature seed: intensity of green colour (characteristic 45)

o) Seed: type of starch grains (characteristic 47)

p) Seed: colour of cotyledon (characteristic 50)

q) Only varieties with plant anthocyanin coloration present: Seed: marbling of testa (characteristic 51)

r) Only varieties with plant anthocyanin coloration present: Seed: violet or pink spots on testa (characteristic 52)

s) Seed: hilum colour (characteristic 53)

t) Seed: weight (characteristic 55)

u) Resistance to *Fusarium oxysporum* f. sp. *pisi* (characteristic 56.1)

v) Resistance to *Erysiphe pisi* Syd. (characteristic 57)

5.4 If other characteristics than those from the Technical Protocol 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.

5.5 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the UPOV-General Introduction to DUS and document TGP/9 "Examining Distinctness".

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 the latter case, the CPVO should be informed.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N°874/2009, to insert additional characteristics and their expressions in respect of a variety.

Technical Protocols with asterisked characteristics (only for certain vegetable species)
In the case of disease resistance characteristics, only those resistances marked with an asterisk (*) in the CPVO column are compulsory.

6.2. 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

Further explanation of the presentation of states of expression and notes is provided in UPOV document TGP/7 "Development of Test Guidelines".

6.3 Example Varieties

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

6.4 Legend

For column 'CPVO N°':

G	Grouping characteristic	-see Chapter 5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	Explanations for individual characteristics	-see Chapter 8.2
(*)	Asterisked characteristic	-see Chapter 6.1.2

For column 'UPOV N°':
The numbering of the characteristics is provided as a reference to the UPOV guideline.
(*) UPOV Asterisked characteristic -Characteristics that are important for the international harmonization of variety descriptions.

For column 'Stage, method':

MG, MS, VG, VS		-see Chapter 4.1.5
(a)-(c)	Explanations covering several Characteristics	-see Chapter 8.1
00-99	Explanations on growth stages	-see Chapter 8.3

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1. (+)	**1. (*)**	**30-240**	**Plant: anthocyanin coloration**		
	QL	**VG**	absent	Avola, Solara	1
G			present	Pidgin, Rosakrone	9
2.	**2.**	**30-240**	**Stem: anthocyanin coloration of axil**		
	QL	**VG**	absent	Avola, Maro	1
			single ring	Assas, Tirabeque	2
			double ring	Caroubel	3
3. (+)	**3. (*)**	**30-199**	**Stem: fasciation**		
	QL	**VG**	absent	Avola, Solara	1
G			present	Bikini, Rosakrone	9
4. (+)	**4. (*)**	**240-250**	**Stem: length**		
	QN	**MS**	very short	Zephir	1
			short	Mini, Nobel	3
			medium	Calibra, Xantos	5
			long	Blauwschokker, Livia	7
G			very long	Mammoth Melting Sugar	9
5. (+)	**5. (*)**	**210-240**	**Stem: number of nodes up to and including first fertile node**		
	QN	**MS**	very few	Kelvil	1
			few	Smart, Zero4	3
			medium	Markana, Susan	5
			many	Cooper	7
G			very many	Regina	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
6.	**6. (*)**	**40-240**	**Foliage: colour**		
	PQ	**VG**	yellow green	Pilot	1
			green	Avola, Paris, Progreta, Waverex	2
			blue green	Polar	3
7.	**7.**	**40-240**	**Only varieties with foliage colour: green (Char.6, state 2) Foliage: intensity of colour**		
	QN	**VG**	light	Paris, Twinkle	3
			medium	Lisa, Rondo	5
			dark	Waverex	7
8.	**8. (*)**	**20-240**	**Leaf: leaflets**		
	QL	**VG**	absent	Hawk, Solara	1
G			present	Avola, Rhea	9
9. (+)	**9.**	**200-240**	**Leaf: maximum number of leaflets**		
	QN	**MS/MG**	few	Jof	3
			medium	Dark Skin Perfection, Finale	5
			many	Ultimo	7
10.	**10.**	**216-226**	**Leaflet: size**		
	QN	**MS/VG**	very small	Payette	1
		(a)	small	Mini	3
			medium	Finale	5
			large	Alderman	7
			very large	Corne de bélier	9
11.	**11.**	**216-226**	**Leaflet: length**		
	QN	**MS/VG**	short	Eagle, Polar	3
		(a)	medium	Bohatyr, Dakota	5
			long	Corne de bélier, Delikata	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
12.	**12.**	**216-226**	**Leaflet: width**		
	QN	**MS/VG**	narrow	Alouette, Grapis	3
		(a)	medium	Dakota, Irina	5
			broad	Adept, Tirabeque	7
13. (+)	**13.**	**216-226**	**Leaflet: position of broadest part**		
	QN	**MS/VG**	at middle or slightly towards base	Nobel, Salome	1
		(a)	moderately towards base	Columbia, Maro	2
			strongly towards base	Griffin, Progreta	3
14. (+)	**14.**	**30-240**	**Leaflet: dentation**		
	QN	**VG**	absent or very weak	Progreta	1
		(a)	weak	Snowflake	3
			medium	Cabree	5
			strong	Amos	7
			very strong	Sugar Star	9
15. (+)	**15. (*)**	**216-226**	**Stipule: length**		
	QN	**MS/VG**	short	Eagle, Steffi	3
		(b)	medium	Timo, Twinkle	5
			long	Alderman, Rhea	7
16. (+)	**16. (*)**	**216-226**	**Stipule: width**		
	QN	**MS/VG**	narrow	Eagle, Steffi	3
		(b)	medium	Timo, Twinkle	5
			broad	Early Onward	7
17.	**17.**	**216-226**	**Stipule: size**		
	QN	**MS/VG**	small	Dakota, Zero4	3
		(b)	medium	Jackpot, Misty	5
			large	Beetle, Early Onward	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
18. (+)	**18**	**216-226**	**Stipule: length from axil to tip**		
	QN	**MS/VG**	short	Fortress, Zero4	3
		(b)	medium	Cabree, Orka	5
			long	Beetle, Early Onward	7
19. (+)	**20. (*)**	**200-240**	**Stipule: flecking**		
	QL	**VG**	absent	Lisa, Tafila	1
G			present	Avola, Maro	9
20. (+)	**21.**	**200-240**	**Stipule: density of flecking**		
	QN	**VG**	very sparse	Progreta	1
			sparse	Backgammon, Waxwing	3
			medium	Accent, Ambassador	5
			dense	Avola, Zelda	7
			very dense	Oregon Sugar Pod	9
21. (+)	**22.**	**216-226**	**Petiole: length from axil to first leaflet or tendril**		
	QN	**MS/VG**	short	Hellas, Keo	3
			medium	Avola, Solara	5
			long	Saskia, Tafila	7
22. (+)	**23.**	**216-226**	**Only varieties with leaflets absent: Petiole: length from axil to last tendril**		
	QN	**MS/VG**	short	Choucas, Frediro	3
			medium	Alambo, Alezan	5
			long	Arosa, Calao	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
23. (+)	**24. (*)**	**214**	**Time of flowering**		
	QN	**MG**	very early	Tempo	1
			early	Smart, Zero4	3
			medium	Carlton, Waverex	5
			late	Cooper, Purser	7
			very late	Livioletta	9
24. (+)	**25. (*)**	**216-226**	**Only varieties with stem fasciation absent: Plant: maximum number of flowers per node**		
	QN	**MS/VG**	one	Progress N°9, Tyla	1
			two	Banff, Cooper	3
			three	Ultimo, Zodiac	5
G			four and more	Amesa, Calibra, Survivor	7
25.	**26. (*)**	**216-218**	**Only varieties with plant anthocyanin coloration present: Flower: colour of wing**		
	PQ	**VG**	white with pink blush		1
		(b)	pink	Rosakrone	2
			reddish purple	Assas	3
26. (+)	**27.**	**216-218**	**Only varieties with plant anthocyanin coloration absent: Flower: colour of standard**		
	PQ	**VG**	white	Gloton, Record	1
		(b)	whitish cream	Cooper, Maro	2
			cream	Orcado	3
27. (+)	**28.**	**216-218**	**Flower: width of standard**		
		MS/VG	narrow	Eagle, Progreta	3
QN		**(b)**	medium	Bikini, Cooper	5
			broad	Pilot, Tafila	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
28. (+)	**29. (*)**	**216-218**	**Flower: shape of base of standard**		
	QN	**VG**	strongly raised		1
		(b)	moderately raised	Progreta	3
			level	Markado, Solara	5
			moderately arched	Avola, Cooper	7
			strongly arched	Bohatyr, Kennedy	9
29.	**31.**	**216-218**	**Flower: width of upper sepal**		
	QN	**VG**	narrow	Abador	3
		(b)	medium	Conservor	5
			broad	Kodiak	7
30. (+)	**32.**	**212-240**	**Flower: shape of apex of upper sepal**		
	PQ	**VG**	acuminate	Dawn	1
		(b)	acute	Kelvedon Wonder	2
			rounded	Kodiak	3
31. (+)	**33.**	**218-245**	**Peduncle: length of spur**		
	QN	**MS/VG**	short	Cabro, Kirio	1
		(b)	medium	Metaxa, Rialto	2
			long	Alezan, Calao	3
32. (+)	**34.**	**235-245**	**Peduncle: length from stem to first pod**		
	QN	**MS/VG**	short	Goblin, Orcado	3
		(c)	medium	Bohatyr, Maro	5
			long	Kabuki, Reveille	7
33. (+)	**35.**	**235-245**	**Peduncle: length between first and second pods**		
	QN	**MS/VS**	short	Alize, Atila	3
		(c)	medium	Kirio	5
			long	Aladin	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
34. (+)	**36.**	**235-245**	**Peduncle: number of bracts**		
	QN	**MS**	absent or few	Fauvette, Kirio	1
		(b)	medium	Delta, Duez	2
			many	Eiffel, Goelan	3
35.	**37. (*)**	**240**	**Pod: length**		
	QN	**MS/VG**	very short	Cepia, Vermio	1
		(c)	short	Progreta, Solara	3
			medium	Cooper, Jof	5
			long	Hurst Green Shaft, Protor	7
G			very long	Tirabeque	9
36. (+)	**38. (*)**	**240**	**Pod: width**		
	QN	**MS/VG**	very narrow	Claire	1
		(c)	narrow	Picar, Ultimo	3
			medium	Progreta, Solara	5
			broad	Finale, Kahuna	7
			very broad	Kennedy	9
37. (+)	**39. (*)**	**310**	**Pod: parchment**		
	QL	**VG**	absent or partial	Sugar Ann	1
G		**(c)**	entire	Avola, Solara	9
38. (+)	**40. (*)**	**240**	**<u>Excluding varieties with pod parchment: entire</u>: Pod: thickened wall**		
	QL	**VG**	absent	Nofila, Reuzensuiker	1
G		**(c)**	present	Cygnet, Sugar Ann	9
39. (+)	**41. (*)**	**240**	**<u>Only varieties with pod: thickened wall: absent</u>: Pod: shape of distal part**		
	QL	**VG**	pointed	Jof, Oskar	1
G		**(c)**	blunt	Avola, Solara	2

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
40. (+)	**42. (*)**	**240**	**Pod: curvature**		
	QN	**VG**	absent or very weak	Finale, Maro	1
		(c)	weak	Eagle, Span	3
			medium	Carlton, Hurst Green Shaft	5
			strong	Delikata, Jof	7
G			very strong	Oskar	9
41. (+)	**43. (*)**	**230-240**	**Pod: colour**		
	PQ	**VG**	yellow		1
		(c)	green	Avola, Solara	2
			blue green	Show Perfection	3
G			purple	Blauwschokker	4
42.	**44.**	**230-240**	**Only varieties with pod colour green (Char. 41, state 2): intensity of green colour**		
	QN	**VG**	light	Solara, Ultimo	3
		(c)	medium		5
			dark	Dark Skin Perfection, Hawaï	7
43. (+)	**45. (*)**	**240-245**	**Excluding varieties with pod parchment: entire: Pod: suture strings**		
	QL	**VG**	absent	Nofila, Sugar Lace	1
		(c)	present	Crispi, Reuzensuiker	9
44. (+)	**46. (*)**	**226**	**Pod: number of ovules**		
	QN	**MS**	few	De Grace, Phoenix	3
		(c)	medium	Backgammon, Hawk	5
			many	Karisma	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
45. (+)	**47. (*)**	**230-240**	**Immature seed: intensity of green colour**		
	QN	**VG**	light	Arabelle, Solara, Ultimo	3
			medium		5
G			dark	Dark Skin Perfection, Hawaii	7
46. (+)	**48.**	**320**	**Seed: shape**		
	PQ	**VG**	ellipsoid	Solara	1
			cylindrical	Span, Timo	2
			rhomboid	Maro, Progreta	3
			irregular		4
47. (+)	**49. (*)**	**320**	**Seed: type of starch grains**		
	QL	**VG**	simple	Adagio, Maro, Solara	1
G			compound	Avola, Polar	2
48. (+)	**50. (*)**	**320**	**Only varieties with seed shape: cylindrical; and type of starch grains: simple: Seed: wrinkling of cotyledon**		
	QL	**VG**	absent	Atila, Paris	1
			present	Allsweet, Zorba	9
49.	**51. (*)**	**320**	**Only varieties with seed: type of starch grains: compound: Seed: intensity of wrinkling of cotyledon**		
	QN	**VG**	weak	Darfon, Zefier	3
			medium	Ziggy	5
			strong	Oskar, Quad	7
			very strong		9
50. (+)	**52. (*)**	**320**	**Seed: colour of cotyledon**		
	PQ	**VG**	green	Avola, Solara	1
			yellow	Caractacus, Hardy	2
G			orange		3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
51.	**53. (*)**	**320**	**<u>Only varieties with plant anthocyanin coloration present</u>: Seed: marbling of testa**		
	QL	**VG**	absent	Rhea, Rif	1
G		**(d)**	present	Assas, Pidgin	9
52.	**54. (*)**	**320**	**<u>Only varieties with plant anthocyanin coloration present:</u> Seed: violet or pink spots on testa**		
	QL	**VG**	absent	Pidgin, Rif	1
		(d)	faint	Assas, Susan	2
G			intense	Arvika, Rhea	3
53. (+)	**55. (*)**	**320**	**Seed: hilum colour**		
	QL	**VG**	same colour as testa	Avola, Solara	1
G		**(d)**	darker than testa	Nofila, Rif	2
54.	**56.**	**320**	**<u>Only varieties with plant anthocyanin coloration present</u>: Seed: colour of testa**		
	PQ	**VG**	reddish brown	Rhea, Rosakrone	1
		(d)	brown	Pidgin	2
			brownish green	Lisa, Susan	3
55. (+)	**57. (*)**	**320**	**Seed: weight**		
	QN	**MG**	very low	Ultimo	1
			low	Hawk	3
			medium	Phoenix, Sugar Flash	5
			high	Kennedy, Maro	7
G			very high	Bamby, Kabuki	9
56. (+)	**58.**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *pisi***		
			Race 1		
QL			absent	Bartavelle	1
G			present	New Era, Nina	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
57. (+)	**59.**	**VG**	**Resistance to *Erysiphe pisi* Syd.**		
	QL		absent	Cabree	1
G			present	Ema, Sugar Bon, Stratagem (JI2302), Vivaldi	9
58. (+)	**60.**	**VG**	**Resistance to *Ascochyta pisi* Race C**		
	QL		absent	Crecerelle, Kelvedon Wonder	1
			present	Madonna, Nina, Rondo	9

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 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) Leaflet: Unless otherwise indicated, all observations should be made on the first leaflet at the second flowering node.

b) Stipule, flower and peduncle: Unless otherwise indicated, all observations should be made at the second flowering node.

c) Pod: Unless otherwise indicated, all observations should be made at the second fertile node.

d) Seed of varieties with plant anthocyanin coloration present contain tannins in the testa, which may darken with age, obscuring the expression of other seed characteristics. Recording of these seed characteristics should be carried out within nine months of harvest; assessment is easiest under conditions of bright natural daylight.

8.2 Explanations for individual characteristics

Ad. 1: Plant: anthocyanin coloration

The anthocyanin coloration should be recorded as present if anthocyanin occurs in one or more of the following: seed, foliage, stem, axil, flower or pod.

Ad. 3: Stem: fasciation

Fasciated stems may be ribbed and flattened up to a width of 3 cm; several apical growing points often result in multiple flowers or pods at the top of the plant.

multiple flowers

ribbed stems

Ad. 4: Stem: length

Only the main stem should be recorded. The observations should be made on harvested plants when seed is green and fully developed. The starting point of the measurement is the first node with 'scale' leaves.

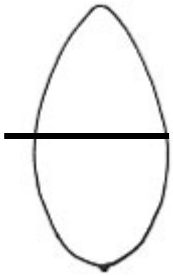
Ad. 5: Stem: number of nodes up to and including first fertile node

Only the main stem should be recorded. The starting point of the measurement is the first node with 'scale' leaves.

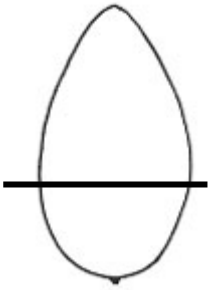
Ad. 9: Leaf: maximum number of leaflets

Assessment should be made over the whole plant.

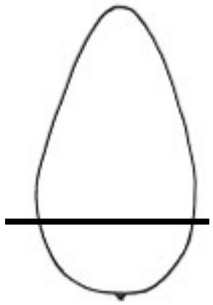
Ad. 13: Leaflet: position of broadest part



1
at middle or slightly towards base



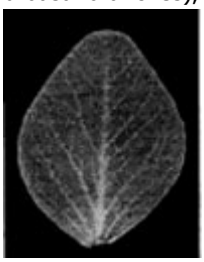
2
moderately towards base



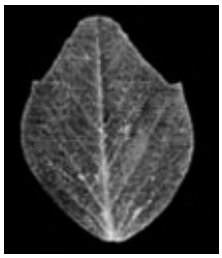
3
strongly towards base

Ad. 14: Leaflet: dentation

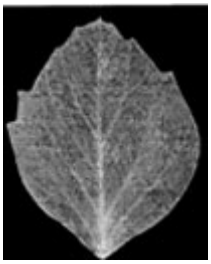
The maximum expression should be recorded; observations should only be made on the main stem (excluding aerial and basal branches), and above node six.



1
absent or very weak



3
weak



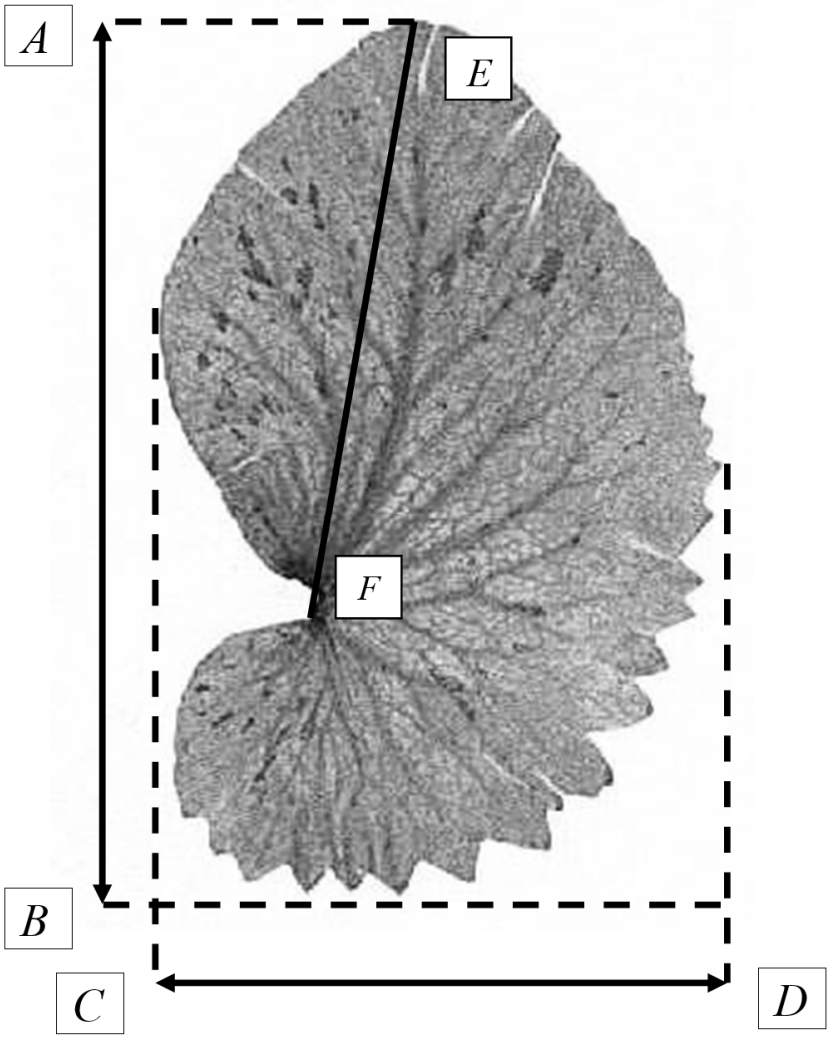
5
medium

7
strong

9
very strong

Ad. 15: Stipule: length
Ad. 16: Stipule: width
Ad. 18: Stipule: length from axil to tip

Observations should be made on stipules which have been detached from the plant and flattened.



Stipule:length (15)	A - B
Stipule: width (16)	C - D
Stipule: length from axil to tip (18)	E - F

Ad. 19: Stipule: flecking
Ad. 20: Stipule: density of flecking

Assessment should be made on the main stem only. The presence of flecking on any stipule on the main stem means that flecking is present. It should be ensured that foliage at the lowest nodes has not senesced before assessment. The plant should have at least eight nodes, since flecking in some varieties may not be expressed at lower nodes. The density of flecking should be observed on the part of the plant with most flecking.

1	3	5	7	9
very sparse	sparse	medium	dense	very dense

Ad. 21: Petiole: length from axil to first leaflet or tendril
Ad. 22: Only varieties with leaflets absent: Petiole: length from axil to last tendril

Petiole length from axil to the first leaflet or tendril (22)	A - B
Total length of petiole including tendrils (23)	A - C

Ad. 23: Time of flowering

The time of flowering is when 30% of plants have at least one flower open.

Ad. 24: Only varieties with stem fasciation absent: Plant: maximum number of flowers per node

Assessment should be made over all flowering nodes on the main stem of the plant. A count is made of the maximum number of flowers at any node on each plant examined. An average is then calculated for the total number of plants examined per plot.

As flower set is dependent on temperature and available soil moisture, it is not unusual to record mean flower numbers between 1, 2 and 3 flowers. Mean values within 0.2 of a whole number should be rounded to that number for descriptive purposes e.g. mean 1.2 will be one flowered (note 1) and 1.8 will be two flowered note 3). All other mean values will fall into the intermediate states e.g. 1.3 or 1.7 will be one to two flowered (note 2).

Ad. 26: Only varieties with plant anthocyanin coloration absent: Flower: colour of standard

The colour of standard should be recorded on flowers which are fully opened and fresh.

Ad. 27: Flower: width of standard

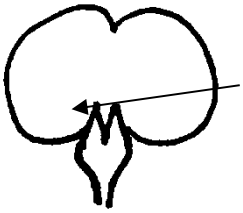
The standard should be detached from the flower and flattened on a hard, flat surface.

Ad. 28: Flower: shape of base of standard

The standard should be detached and flattened on a hard, flat surface.

1 strongly raised | 3 moderately raised | 5 level | 7 moderately arched | 9 strongly arched

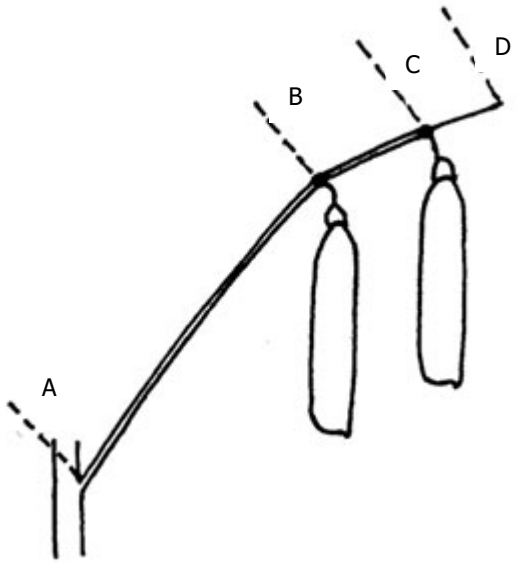
Ad. 30: Flower: shape of apex of upper sepal



back of flower standard

1 acuminate | 2 acute | 3 rounded

Ad. 31: Peduncle: length of spur
Ad. 32: Peduncle: length from stem to first pod
Ad. 33: Peduncle: length between first and second pods



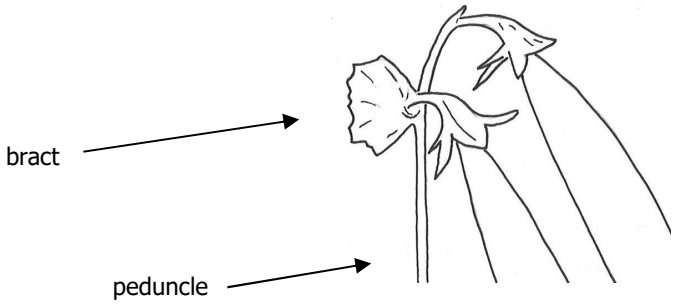
A – B = Peduncle: length from stem to first pod (34)

B – C = Peduncle: length between first and second pods (35)

C – D = Peduncle: length of spur (33)

Ad. 34: Peduncle: number of bracts

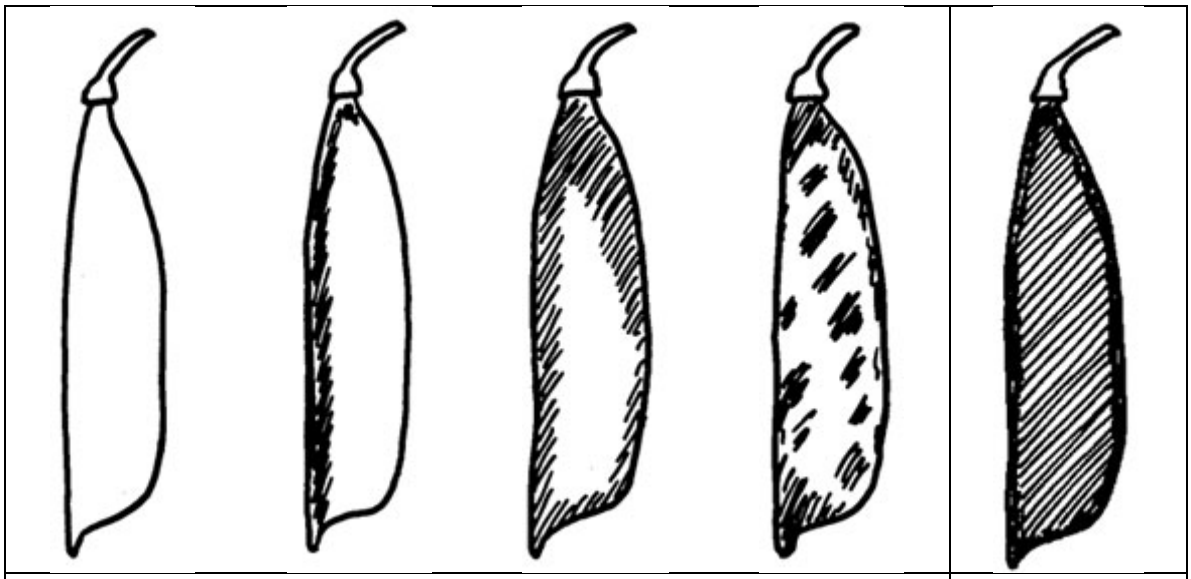
Bracts are modified leaves which occur on the peduncle. The number of bracts is calculated on the basis of averages across plants.



Ad. 36: Pod: width

The observations should be made on well-developed green pods; the width is assessed from suture to suture on unopened pods.

Ad. 37: Pod: parchment
(viewed on the inside of the pod wall)



1 absent or partial	2 entire

(1) The observations should be made on dry pods with the exception of 'Snap Peas'. Snap Peas (Sugar Peas with thickened pod walls) are best recorded when green, in order to minimize fungal infection which can prevent observation of the parchment.

(2) The pod should be opened along the suture without damaging the edges of the two sides of the pod. The distribution of sclerenchyma, which makes up the parchment, may either be observed by staining (a drop of Phloroglucinol dissolved in Ethanol followed by a drop of concentrated (37%) Hydrochloric Acid), or by reflecting light (preferably daylight) on the inside of the pod wall.

(3) In the case of varieties with the state "entire", the parchment will occur as a thick layer in all pods.

Ad. 38: Excluding varieties with pod parchment: entire: Pod: thickened wall

The observations should be made on well-developed pods not showing any signs of senescence. Unopened harvested pods should be cut in cross section to examine pod wall thickness.

1
absent

9
present

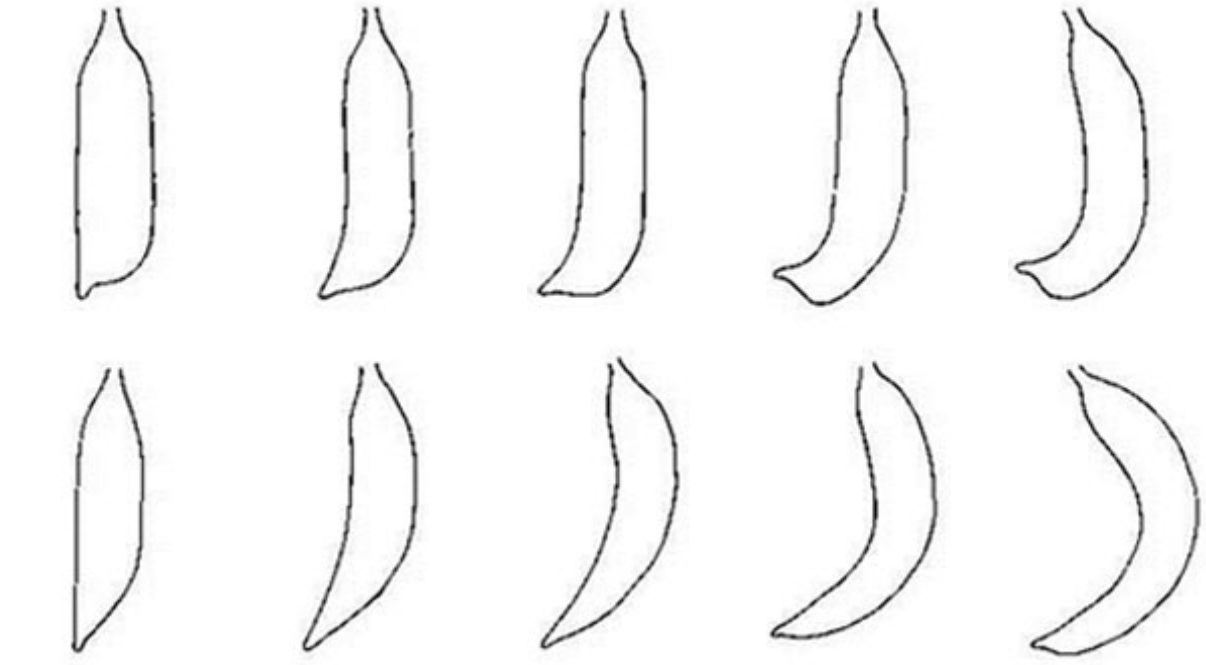
Ad. 39: Only varieties with Pod: thickened wall: absent: Pod: shape of distal part

Observations should be made on several nodes of each plant when pods are fully developed, but before any senescence.

1
pointed

2
blunt

Ad. 40: Pod: curvature



1	3	5	7	9
absent or very weak	weak	medium	strong	very strong

Ad. 41: Pod: colour

Green pods may be pale or dark, the colour is correlated with pale or dark immature seed colour.
Blue green pods are dark and slightly bluish. The colour develops with time, and may be more accentuated in hotter, drier conditions.
Purple pods may be entirely purple or partially purple; occasionally the amount and distribution of anthocyanin may vary within the plant.

Ad. 43: Excluding varieties with pod parchment: entire: Pod: suture strings

When temperatures exceed 20° C, the formation of suture strings is delayed. Observations should be made on fully developed pods.
Varieties with rudimentary suture strings are considered as state "absent".

Ad. 44: Pod: number of ovules

The number of ovules is best recorded when the pods are flat. The number of ovules should be observed before seed development.

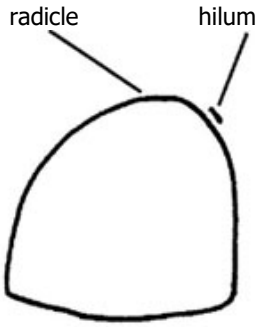
Ad. 45: Immature seed: intensity of green colour

Immature seed colour in some varieties with green cotyledons may appear creamy white before the seed is fully developed. Observations should be made on fully developed, fresh seed in a side-by-side comparison with example varieties.

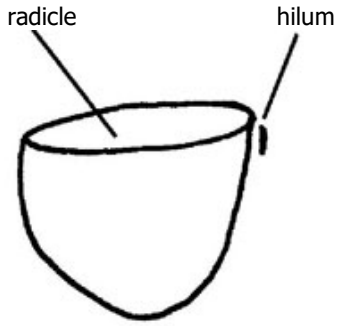
Ad. 46: Seed: shape

Seeds which grow nearest the peduncle end or the distal end of the pod ('end seeds') are rounded on the radicle or the distal (opposite to the radicle) surfaces and should be excluded before shape is assessed. 'Golf ball' and other irregular dimpling should be ignored.

Orientate the seed so that the hilum is at the upper right hand side with radicle on top.



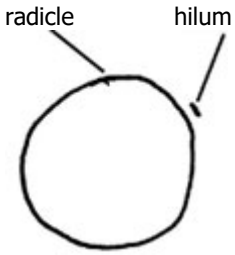
If the seed is rounded on the radicle surface only, it is an end seed growing nearest the peduncle end of the pod.

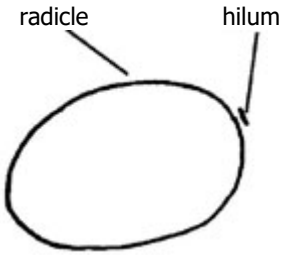


If the seed is rounded on the distal surface only, it is an end seed growing nearest the distal end of the pod.

1. Ellipsoid

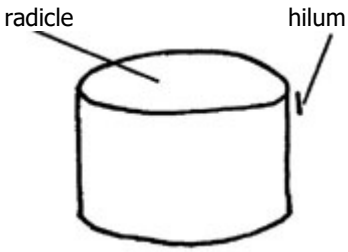
Seeds with no, or very weak, compression on the radicle and/or the distal surfaces.

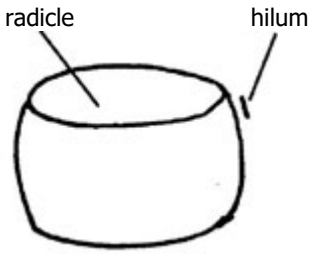




2. Cylindrical

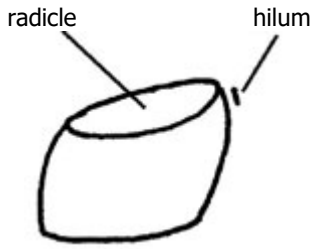
Seeds compressed on the radicle and distal surfaces. Square to rectangular or with rounded sides in longitudinal section.





3. Rhomboid

Seeds irregularly compressed on the radicle and distal surfaces, but also irregularly compressed on the abaxial surfaces.



4. Irregular

Seeds irregularly compressed; not one of the above shapes.

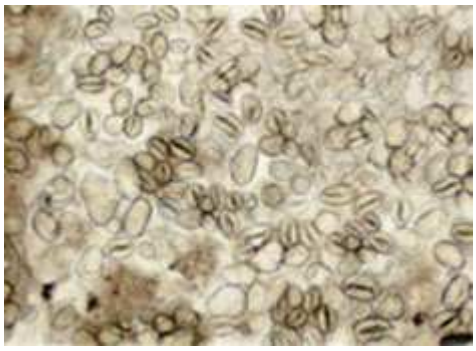
Ad. 47: Seed: type of starch grains

(1) Following the removal of the testa, fine fragments of tissue should be extracted from the cotyledon and placed on a microscope slide. A droplet of water is added to the extracted tissue and another microscope slide is placed on top. The tissue and water mixture is then squashed gently between the two slides. Too much pressure during squashing results in fragmentation of the grains, too little pressure will not provide a layer thin enough for examination.

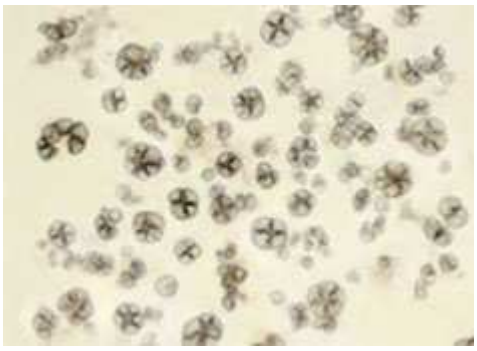
(2) A microscope with transmitted light, using X16 eye-pieces and either X10 or X40 objectives, is most suitable for examination. For examination of compound grains the larger objectives will be required.

(3) Simple grains resemble wheat seeds or coffee beans in shape, often with what looks like a suture line running along their length.

(4) Compound grains look irregularly star-shaped and appear to be made of a number of segments. The centre of the grains may appear cross-shaped. In varieties with high sweetness, compound starch grains are very small and few in number.



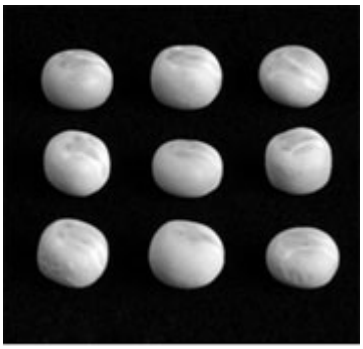
1
simple



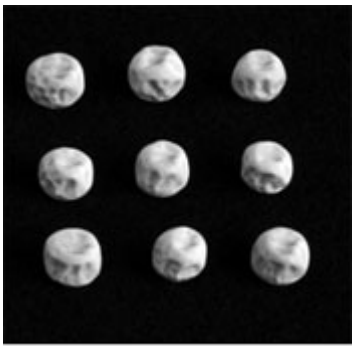
2
compound

Ad. 48: Only varieties with seed shape: cylindrical; and type of starch grain: simple: Seed: wrinkling of cotyledon

'Golf ball' and large dimples should be ignored in the assessment of cotyledon wrinkling.



1
absent



9
present

Ad. 50: Seed: colour of cotyledon

Following the removal of the testa, the seed is cut along the line of the cotyledon suture. Assessment of both external (abaxial) and internal (adaxial) surfaces of the cotyledon may be necessary. Immature seeds should be excluded from the assessment.

The expression varies with environmental conditions:

- bleaching, caused by sunlight or chemical changes in the plant, can remove colour from seeds making it difficult to determine cotyledon colour; cutting the seed in half enables the assessment of the internal colour which may be less affected.
- colour becomes dull with age, even if seed is stored in cold, dark conditions.
- colour can darken in the presence of high amounts of Tragacanth oil occurring on the underside of the testa. This fades as the seed ages. Seeds with tannin may darken with age.
- orange cotyledons can be difficult to determine without reference to an example variety.

Ad. 53: Seed: hilum colour

The hilum area should be lightly polished with a cloth before recording, to remove any loose tissue present. In varieties with plant anthocyanin present, the testa will contain tannins which vary in colour from reddish brown to brown to brownish green. Where the hilum colour is darker than the testa, melanin pigment is expressed as a black or dark brown colour. It can be difficult to assess hilum colour if the testa tannins darken with age; assessment should therefore be made within nine months of seed harvest.

Ad. 55: Seed: weight

Seed weight should be measured on at least two samples of 100 seeds. Immature and infected seeds should be excluded.

Ad. 56: Resistance to *Fusarium oxysporum* f. sp. *pisi* race 1 (Near wilt)

1. Pathogen..... *Fusarium oxysporum* f. sp. *pisi* race 1
2. Quarantine status ..... no
3. Host species ..... Pea – *Pisum sativum* L.
4. Source of inoculum ..... For Fop: 1, GEVES[1] (FR), INIA[2] (SP) or SASA[3] (UK)
5. Isolate ..... *Fusarium oxysporum* f. sp. *pisi* race 1 strain MATREF 04-02-01-01 (the test protocol has been validated in a CPVO co-funded project[4] with this isolate/race).
6. Establishment isolate identity ..... genetically defined pea controls (See ISF website: https://www.worldseed.org/wp-content/uploads/2019/09/Pea-near-wilt_July2019_Final.pdf))

Differentials host	Race (ISF Code)
	1 (Fop: 1)
Little Marvel, M410	S
Dark Skin Perfection, Vantage	HR
Mini	S
New Era, Mini 93	HR

HR = highly resistant; S = susceptible

7. Establishment pathogenicity..... Test on susceptible plants
8. Multiplication inoculum
8.1 Multiplication medium..... Multiplication on agar medium: malt Agar or PDA for example
8.2 Multiplication variety..... -
8.3 Plant stage at inoculation..... -

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8.4 Inoculation medium..... Multiplication on agar medium: water for scraping agar plates
Multiplication on liquid medium: Potato Dextrose Broth, Kerrs broth or Czapek-Dox (3 to 7 days old aerated culture) for example.
8.5 Inoculation method ..... -
8.6 Harvest of inoculum ..... see 10.1
8.7 Check of harvested inoculum..... see 10.2.
8.8 Shelf life/viability inoculum..... 4/8h, keep cool to prevent spore's germination. More than 3 years at -20°C.
9. Format of the test
9.1 Number of plants per genotype ..... At least 20 inoculated plants and 5 non inoculated plants per variety.
9.2 Number of replicates ..... -
9.3 Control varieties..... Susceptible controls: Bartavelle
Resistant controls: New Era, Nina
9.4 Test design..... -
9.5 Test facility..... Climate room or greenhouse.
9.6 Temperature..... 20-25°C
9.7 Light ..... 12 hours or longer
9.8 Season..... -
9.9 Special measures ..... it is important to compare all plants with a negative control of the same sample to allow to interpret symptoms of root rot or senescence or 'wilting' due to the stress of having roots cut and not due to *F. oxysporum* infection.
10. Inoculation
10.1 Preparation inoculum ..... Filter through muslin.
10.2 Quantification inoculum..... 10^6 spores/mL
10.3 Plant stage at inoculation ..... seeds or 2 weeks old seedlings (2-3 node stage).
10.4 Inoculation method..... For seeds: sowing in contaminated substrate (soil based substrate), 750mL of suspension of spores at 10^6sp/mL for 5L of substrate.
For 2 weeks seedlings:
Sowing in a mix of vermiculite + soil or soil based substrate
Cut roots, dip in the spores suspension for 1 to 5 minutes and transplant in soil based substrate in a new tray
10.7 Final observations..... 28 days post-inoculation.
11. Observations
11.1 Method..... Visual
11.2 Observation scale ..... 0: no symptoms or equivalent to resistant control, 1 or 2 senesced lower leaves and slight reduction in growth compared to resistant control of same variety are acceptable
1: Range from a few chlorotic or wilting/senesced leaves not present on, or more than on the resistant control, up to many leaves with symptoms of senescence or wilting; some leaf drop, upper part of the plant still green and growing
2: Range from most of the plant senesced or wilted but still alive, to plants brown and dead with stem collapsed
Notes 0 and 1 are resistant.
Note 2 is susceptible


note 0
resistant


note1
resistant


note 2
susceptible


note 2
susceptible

Varieties with the same or higher level of resistance as New Era will be interpreted as resistant. Varieties with a lower level of resistance than New Era will be interpreted as susceptible. Nina will be highly resistant, Bartavelle will be highly susceptible. . New Era expresses weak symptoms and variation can occur in these weak symptoms depending on the agressivity of the test conditions.

11.3 Validation of test ..... evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.

12. Interpretation of data in terms of UPOV characteristic states

absent ..... [1] susceptible
present ..... [9] resistant

13. Critical control points ..... each lab has to define the best method of inoculation in its lab depending on controls results.
Inoculation by sowing in contaminated soil can in some cases lead to germination problems. No conclusion can be done in this case, and the test should be repeated.

Ad. 57: Resistance to *Erysiphe pisi* Syd. (Powdery Mildew)

1.	Pathogen	Powdery mildew – *Erysiphe pisi*
2.	Quarantine status	No
3.	Host species	Pea – *Pisum sativum* L
4.	Source of inoculum	GEVES[5] (FR)
5.	Isolate	*Erysiphe pisi e.g.* isolate 2430 The test protocol has been validated in a CPVO co-funded project[6] with this isolate/race
6.	Establishment isolate identity	Validation by use specific EryF/EryR primers to validate the species of *Erysiphe* (use ITS primers from Attanayake et al, 2010[7].)
7	Establishment pathogenicity	use susceptible variety (e.g. Aladin, Cabree or Ottoman)
8.	Multiplication inoculum	
8.1	Multiplication medium	living plant
8.2	Multiplication variety	see 7
8.3	Plant stage at inoculation	see 10.3

[5] GEVES; matref@geves.fr
[6] Harmores 3 CPVO project (*insert link to the final report on CPVO website when available*)
[7] Attanayake et al, 2010. *Erysiphe trifolii*– a newly recognized powdery mildew pathogen of pea, Plant Pathology, Volume 59, Issue 4, p712-720

8.5	Inoculation method	see 10.4
8.6	Harvest of inoculum	For spraying by washing off with demineralized water For sprinkling by detaching leaves of a susceptible host plant
8.7	Check of harvested inoculum	visual check for presence of sporulation
8.8	Shelf life/viability inoculum	1-2 hours
9.	Format of the test	
9.1	Number of plants per genotype	20 plants
9.2	Number of replicates	-
9.3	Control varieties	Susceptible: For vegetable crops: Cabree For agricultural crops: Aladin, Ottoman Resistant: For vegetable crop: Ema, Sugar Bon, Vivaldi, Stratagem (JI2302) For agricultural crop: Alezan
9.4	Test design	No non-inoculated control due as it is impossible to place them exactly in the same conditions (risk of contamination).
9.5	Test facility	greenhouse or climate room
9.6	Temperature	20°C (+5°C) but it is advised not to go below 18°C. In some conditions it has been observed that increasing the day temperature up to 27°C allowed a good sporulation on the susceptible control.
9.7	Light	at least 12 h per day
10	Inoculation	
10.1	Preparation inoculum	By spraying: Washing off from leaves by vigorous shaking in a closed container containing water. Sieve the suspension through muslin cloth. By sprinkling: Selection of leaves with strong sporulation.
10.2	Quantification inoculum	By spraying: Counting spores; spores density should be 1.10^5 to 1.10^6 spores/mL By sprinkling: Approximately 1 well-sporulating plant to inoculate 10 plants.
10.3	Plant stage at inoculation	3-4 leaf stage
10.4	Inoculation method	By spraying: Spraying of the suspension of spores on leaves By sprinkling: Shaking of sporulated leaves above the plants to be contaminated.
10.7	End of test	Between 14-21 dpi, when sporulation is well expressed on the susceptible control
11.	Observations	
11.1	Method	visual
11.2	Observation scale	

Susceptible: sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant)				
Resistant: No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms				
Symptoms which should not be confused with *E. pisi*: senescence of older leaves, yellowing, discoloration of leaves and insect damages	 senescing	 yellowing	 discoloration	 insect damage

11.3	Validation of test	Analysis of results should be calibrated with results of R and S controls.
11.4	Off-types	-

12.	Interpretation of data in terms of UPOV characteristic states	Absent (susceptible) Present (resistant)	[1] sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant) [9] No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms
13.	Critical control points	Watering on the substrate (no spraying) to avoid washing the spores off the surface of the leaves. It is not possible to freeze spores. Need to maintain on plants	

Ad. 58: Resistance to *Ascochyta pisi*, Race C (Ascochyta Leaf and Pod Spot)

1. Pathogen..... *Ascochyta pisi*
2. Quarantine status ..... no
3. Host species ..... Pea – *Pisum sativum* L.
4. Source of inoculum ..... GEVES[8] (FR) or SASA[9] (UK)
5. Isolate ..... *Ascochyta pisi* race C strain 21A.13. (the test protocol has been validated in a European CPVO co-funded project[10] with this isolate).
6. Establishment isolate identity ..... genetically defined pea controls (Physiological races of *A. pisi* and differentials, adapted from Gallais et Bannerot, 1992)

Physiological races (Dr Hubbeling) Strains	D N°1	_ Several isolates	_ N°4	_ N°14	C Tézier 21A.13	B _	E _
Gullivert	R	R	R	R	S	R	R
Rondo	R	R	S	VLS	R	R	S
Finale	R	R	S	LS	R	-	-
Kelvedon Wonder	R	S	S	S	S	R	R
Dark Skin Perfection	S	S	S	S	S	R	S
Arabal, Cobri, Starcovert, Sucovert, Vitalis	S	S	S	S	S	S	S

R = resistant; S = susceptible, VLS = very lightly susceptible, LS = lightly susceptible

7. Establishment pathogenicity..... Test on susceptible plants
8. Multiplication inoculum
8.1 Multiplication medium..... V8 agar or Mathur medium or Potato Dextrose Agar or a synthetic medium.
8.2 Multiplication variety..... -
8.3 Plant stage at inoculation..... -
8.4 Inoculation medium..... water, option: add Tween 80 (wetting agent to aid dispersal of spores, e.g. 0.4%)
8.5 Inoculation method ..... -
8.6 Harvest of inoculum ..... see 10.1
8.7 Check of harvested inoculum..... see 10.2.
8.8 Shelf life/viability inoculum..... 4/8h, keep cool to prevent spores' germination
9. Format of the test
9.1 Number of plants per genotype ..... At least 20 plants and 5 non inoculated plants per variety.
9.2 Number of replicates ..... -
9.3 Control varieties..... Susceptible controls: Crecerelle, Kelvedon Wonder
Resistant controls:
Madonna, Nina, Rondo
9.4 Test design..... -
9.5 Test facility..... Climate room or greenhouse.
9.6 Temperature..... 20°C

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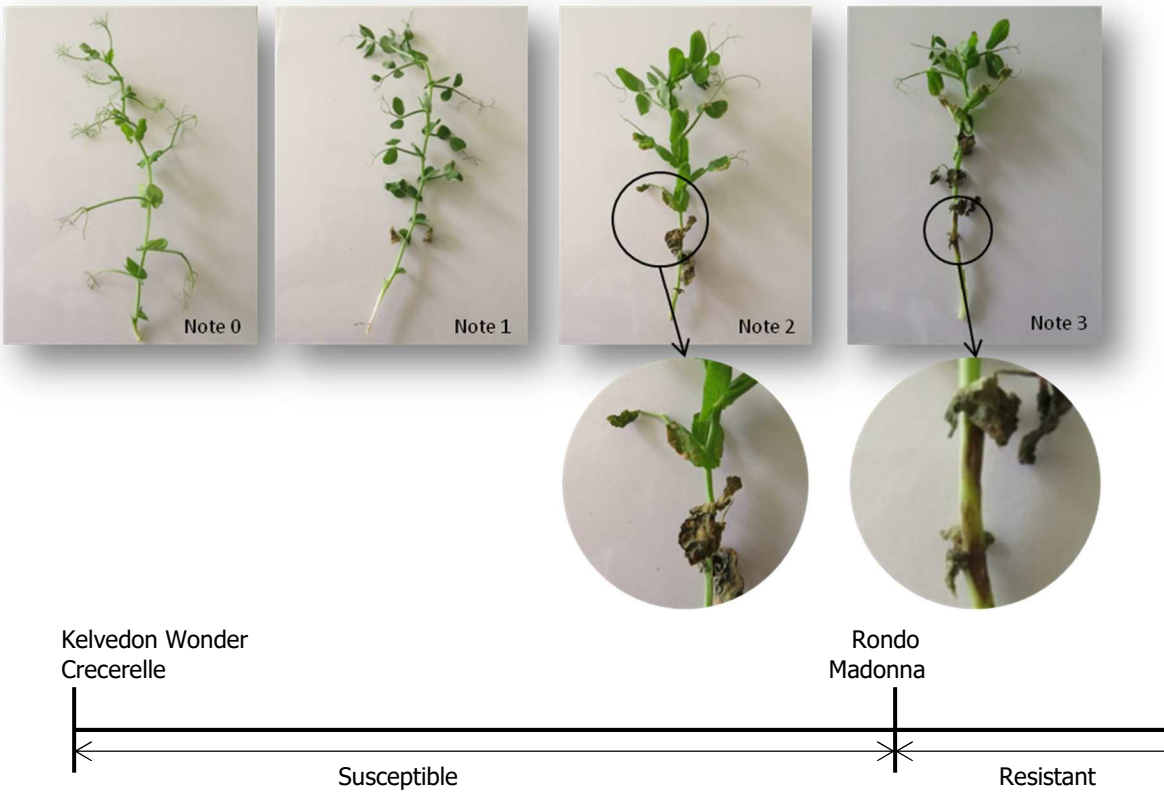
9.7 Light ..... 12 hours or longer
9.8 Season ..... -
9.9 Special measures ..... high humidity or watering by spraying 2 or 3 times per day
10. Inoculation
10.1 Preparation inoculum ..... remove hyphen fragments by straining solution through muslin.
10.2 Quantification inoculum..... 10^6 spores/mL (to adapt depending conditions of tests)
10.3 Plant stage at inoculation ..... 2 weeks old seedlings (i.e. 2-3 node stage)
10.4 Inoculation method..... spraying on green leaves without surface moisture.
10.7 Final observations..... 10-18 days post-inoculation.
11. Observations
11.1 Method..... Visual
11.2 Observation scale ..... 0: no symptoms

1: few small superficial necrosis
2: bigger darker and deep necrosis
3: necrosis at each level of the plant or serious symptoms surrounding the stem
Madonna, Nina and Rondo will be resistant controls; varieties with same level of resistance as Madonna/Rondo and/or Nina will be interpreted as resistant.
Crecerelle and Kelvedon Wonder will be susceptible controls, varieties with a lower level of resistance than Nina as well as Madonna/Rondo will be interpreted as susceptible.



11.3 Validation of test ..... evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.
12. Interpretation of data in terms of UPOV characteristic states

absent	[1]	susceptible
present	[9]	resistant

13. Critical control points ..... -.

8.3 Explanations on growth stages

Key	General Description
0	Germination
00	Dry seed
10	Seedling growth
16	Young seedling with first scale leaf developed
18	Young seedling with second scale leaf developed
20	First pair of stipules at the third node fully opened
22	Stipules at the fourth node fully opened
25	Stipules at the fifth node fully opened
28	Stipules at the sixth node fully opened
30	Vegetative growth
31	Stipules at the seventh node fully opened
34	Stipules at the eighth node fully opened
40	Stipules at the tenth node fully opened
n	Stipules at the Nth node fully opened
200	Reproductive stage
200	Initiation of first flower
206	Development of first flower bud enclosed in stipules
208	Development and sometimes elongation of peduncle
210	Emergence of first flower bud from stipules
212	Emergence of standards from the calyx
214	Opening of the standards and emergence of the wings
216	Slight opening of the wings to show the keel
218	Standards usually fully opened
220	Standards beginning to crumple at the margins
222	Standards and wings showing signs of withering
224	Emergence of the first flat pod
226	Elongation of the flat pod with clearly visible ovules
230	Swelling of the ovules and slight swelling of the pod wall
235	Green seed rounded becoming slightly firm; pods almost fully swollen or developed
240	Green seed firm, becoming starchy; pods fully developed or swollen
245	Green seed becoming pale, testas tough; pod beginning to lose colour
250	Stem and lower foliage becoming yellowish
255	Seed drying and becoming yellowish green; pod becoming wrinkled
260	Lower foliage becoming dry at margins
265	Seed yellowish green; pods wrinkled, pale green
270	Lower foliage becoming dry and papery
275	Seed yellowish-white and rubbery; pods wrinkled and yellowish-green

Key	General Description
280	Stem drying out, becoming yellowish-green
285	Lowest pods yellowish-brown, dry and papery
290	Stem becoming stiff and brittle and appearing yellowish-white
300	Lower and middle nodes with dry papery foliage; lower pods dry and papery
305	All nodes with dry papery foliage; lower and middle pods dry and papery
310	All nodes with dry papery foliage and pods; seed drying but not hard
320	Hard dry seed

9. LITERATURE

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/007/2 Rev. 3 – *Pisum sativum* L. - pea