UPOV
International Union for the Protection of New Varieties of Plants

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PARTIAL REVISION OF THE TEST GUIDELINES FOR PEA

Document prepared by an expert from the European Union

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1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Pea (*Pisum sativum* L.) (document TG/7/10 Rev.).

2. The Technical Working Party for Vegetables (TWV), at its fifty-first session, held in Roelofarendsveen, Netherlands, from July 3 to 7, 2017, considered a proposal for a partial revision of the Test Guidelines for Pea (*Pisum sativum* L.) on the basis of documents TG/7/10 Rev. and TWV/51/6 "Partial Revision of the Test Guidelines for Pea" and proposed the following revisions to the Test Guidelines for Pea (see document TWV/51/16 "Report", paragraphs 104 and 105):

(a) To add new example varieties for Characteristic 60 "Resistance to *Ascochyta pisi* Race"
(b) To change the methodology for Characteristic 60 under Ad. 60

3. The proposed changes are presented below in highlight and underline (insertion).

Proposed change to add new example varieties for Characteristic 60 "Resistance to Ascochyta pisi Race"

Current wording

60. **(+)**	**VG**	**Resistance to *Ascochyta pisi*, Race C**	**Résistance à *Ascochyta pisi*, race C**	**Resistenz gegen *Ascochyta pisi*, Pathotyp C**	**Resistencia a *Ascochyta pisi*, Raza C**		
QL		absent	absente	fehlend	ausente	Kelvedon Wonder	1
		present	présente	vorhanden	presente	Rondo	9

Proposed new wording

60. **(+)**	**VG**	**Resistance to *Ascochyta pisi*, Race C**	**Résistance à *Ascochyta pisi*, race C**	**Resistenz gegen *Ascochyta pisi*, Pathotyp C**	**Resistencia a *Ascochyta pisi*, Raza C**		
QL		absent	absente	fehlend	ausente	Crecerelle, Kelvedon Wonder	1
		present	présente	vorhanden	presente	Madonna, Nina, Rondo	9

Proposed change to the methodology for Characteristic 60 under Ad. 60

Current wording

Ad. 60: Resistance to *Ascochyta pisi*, Race C (Ascochyta Leaf and Pod Spot)

Resistant and Susceptible varieties
Kelvedon Wonder (susceptible = resistance absent (1))
Rondo (resistant = resistance present (9))
Isolates and isolate identity
Isolate used in the test: Tezier Strain
Isolate identity is determined by testing against a host differential set.

Maintenance of isolates
Maintain on Mathur medium at ambient temperature. Isolate identity is determined by testing against a host differential set.

Source for isolates: GEVES SNES
Station Nationale d'Essais de Semences
Rue George Morel, B.P.24
49071 Beaucouzé Cedex France

Preparation of inoculum
Add 0.4% Tween 80 wetting agent to aid dispersal of spores. Remove hyphal fragments by straining solution through muslin. Concentration of 10^6 spores/ml

Inoculation and assessment of disease
Grow seedlings in glasshouse under natural daylength at 20^{o}C and high humidity. Spray inoculum on young seedlings 10-15 days after emergence; mist spray 2 or 3 times per day for 15 minutes. Alternatively, inoculation can be made at the apex of enclosed leaves. This method does not require conditions of high humidity.

Plants are assessed about 5 days after inoculation. Infection is very clear when present: necrotic lesions are slightly sunken, brown and sharply delineated. Lesions are circular on pods and elongated on stems. Two replicates of 10 plants are grown; a third replicate is grown if any problems arise.

Genetic background
The expression of resistance to Race C (also known as BP2) is controlled by a single dominant gene Rap2. At least five pathotypes and four resistance alleles are known.

Proposed new wording

Ad. 60: Resistance to *Ascochyta pisi*, Race C

1.	Pathogen	*Ascochyta pisi*
2.	Quarantine status	no
3.	Host species	Pea – *Pisum sativum* L.
4.	Source of inoculum	GEVES[1] (FR) or SASA[2] (GB)
5.	Isolate	*Ascochyta pisi* race C strain 21A.13. (the test protocol has been validated in a European CPVO co-funded project[3] 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	Shelflife/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	Crecerelle, Kelvedon Wonder
	Resistant	Nina and Madonna or Rondo
9.4	Test design	-
9.5	Test facility	climate room or greenhouse
9.6	Temperature	20°C
9.7	Light	12 hours or longer
9.8	Season	-

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[3] Harmores 2 CPVO project (http://www.cpvo.europa.eu/main/en/home/documents-and-publications/technical-projects-reports)

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
10.5	First observation	-
10.6	Second observation	-
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
11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	
	absent [1] present [9]	susceptible resistant
13.	Critical control points	-

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