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PARTIAL REVISION OF THE TEST GUIDELINES FOR PEA

Document prepared by an expert from France

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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, agreed that the proposed partial revision of Characteristic 58 "Resistance to *Fusarium oxysporum* f. sp. *pisi"* and its explanation Ad. 58 needed further clarification and therefore should be reconsidered by the TWV at its fifty-second session in 2018.

3. The following changes are proposed:

(a) To change the example varieties for Characteristic 58 "Resistance to *Fusarium oxysporum* f. sp. *pisi* Race 1"
(b) To change the methodology for Characteristic 58 under Ad. 58

4. The proposed changes are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

Proposed change to the example varieties for Characteristic 58 “Resistance to *Fusarium oxysporum* f. sp. *pisi* Race 1”

Current wording

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
58. **(+)**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *pisi***	**Résistance à *Fusarium oxysporum* f. sp. *pisi***	**Resistenz gegen *Fusarium oxysporum* f. sp. *pisi***	**Resistencia a *Fusarium oxysporum* f. sp. *pisi***		
58.1		**Race 1**	**Race 1**	**Pathotyp 1**	**Raza 1**		
QL		absent	absente	fehlend	ausente	Eden, Mammoth Melting Sugar	1
		present	présente	vorhanden	presente	Solara, Twinkle	9
58.2		**Race 5**	**Race 5**	**Pathotyp 5**	**Raza 5**		
QL		absent	absente	fehlend	ausente	Legacy, Little Marvel	1
		present	présente	vorhanden	presente	Serge, Sundance	9
58.3		**Race 6**	**Race 6**	**Pathotyp 6**	**Raza 6**		
QL		absent	absente	fehlend	ausente	Little Marvel, Serge	1
		present	présente	vorhanden	presente	Sundance	9

Proposed new wording

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
58. **(+)**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *pisi***	**Résistance à *Fusarium oxysporum* f. sp. *pisi***	**Resistenz gegen *Fusarium oxysporum* f. sp. *pisi***	**Resistencia a *Fusarium oxysporum* f. sp. *pisi***		
58.1		**Race 1**	**Race 1**	**Pathotyp 1**	**Raza 1**		
QL		absent	absente	fehlend	ausente	~~Eden, Mammoth Melting Sugar~~ Bartavelle	1
		present	présente	vorhanden	presente	~~Solara, Twinkle~~ New Era, Nina	9
58.2		**Race 5**	**Race 5**	**Pathotyp 5**	**Raza 5**		
QL		absent	absente	fehlend	ausente	~~Legacy,~~ Little Marvel, Mini	1
		present	présente	vorhanden	presente	~~Serge,~~ Sundance II	9
58.3		**Race 6**	**Race 6**	**Pathotyp 6**	**Raza 6**		
QL		absent	absente	fehlend	ausente	Little Marvel, ~~Serge,~~ Mini	1
		present	présente	vorhanden	presente	~~Sundance~~ Grant	9

Proposed change to the methodology for Characteristics 58 under Ad. 58

Current wording

Ad. 58.1, 58.2, 58.3: Resistance to *Fusarium oxysporum* f. sp. *pisi*

Resistant and Susceptible varieties
Race 1: Eden, Mammoth Melting Sugar (susceptible = resistance absent (1))
Solara, Twinkle (resistant = resistance present (9))

Race 5: Little Marvel, Legacy (susceptible = resistance absent (1))
Serge, Sundance (resistant = resistance present (9))

Race 6: Little Marvel, Serge (susceptible = resistance absent (1))
Sundance (resistant = resistance present (9))

Isolates and isolate identity
Isolate identity is determined by testing against the host differential set described by Haglund and Kraft (1979). All isolates are derived from single spore cultures.

Isolates used in the test: Race 1: IPO culture collection no. 20379
Race 5: IPO culture collection no. 10279
Race 6: WSU culture type 6

Maintenance of isolates
Maintain in a refrigerator at 4°C as a soil culture (loam) and pass through a susceptible variety every 2-3 years. Isolate identity is determined by testing against a host differential set.

Source for isolates

Races 1 and 5	Research Institute for Plant Protection (IPO) PO Box 9060 NL-6700 GW Wageningen The Netherlands
Race 6	Washington State University (WSU), Research and Extension Unit, Mount Vernon, Washington 98273, United States of America

Preparation of inoculum and assessment of disease
Cultures of the fungus are grown in liquid Czapek-Dox medium at 2°C in daylight conditions for 7 days. The liquid is continuously aerated by sterile air. The cultures are strained through muslin followed by centrifugation at 3,500 rpm for 10 minutes; the solution is diluted with distilled water to a concentration of 10^6 spores/ml.

Inoculation and assessment of disease Test plants and controls are raised in 8 liters of 1:1 peat and sand mixture and adjusted to pH 5.0. 1 liter of spore suspension is used. Two replicates of 10 plants are grown for assessment; a third replicate is grown if any problems arise.

After 3 weeks, or 4 - 5 node stage, the basal third of the seedling roots can be cut and dipped into the inoculum for 3-5 seconds before being transplanted. Four weeks after inoculation, surviving seedlings are recorded as resistant.

Composition of the Czapek-Dox liquid medium

2.0 g	Sodium Nitrate
0.5 g	Potassium Chloride
1.0 g	Dipotassium Phosphate
0.5 g	Magnesium Sulphate
0.01 g	Ferrous Sulphate
30.0 g	Saccharose

The above mixture is added to 1 liter of distilled water and poured into a flask; the solution is sterilized in an autoclave at 115°C for 20 minutes.

Genetic background
A single dominant gene Fw confers resistance to Race 1.

Proposed new wording

Ad. 58.1, 58.2 and 58.3: Resistance to *Fusarium oxysporum* f. sp. *pisi* race 1 (Near wilt) race 5 and race 6

1.	Pathogen	*Fusarium oxysporum* f. sp. *pisi (*race 1, race 5 and race 6)
2.	Quarantine status	no
3.	Host species	Pea – *Pisum sativum* L.
4.	Source of inoculum	For Fop: 1, GEVES[1] (FR), INIA[2] (ES) or SASA[3] (GB) For Fop: 5 and Fop: 6 (*NL will provide the source, probably WSU*)
5.	Isolate	*Fusarium oxysporum* f. sp. *pisi* race 1 strain MATREF 04-02-01-01 (the test protocol has been validated with this isolate/race). *Fusarium oxysporum* f. sp. *pisi* race 5 *Fusarium oxysporum* f. sp. *pisi* race 6
6.	Establishment isolate identity	genetically defined pea controls (See ISF website: http://www.worldseed.org/cms/medias/file/TradeIssues/DiseasesResistance/Differentials/Pea-near_wilt_2012.pdf)

Differentials host	Race (ISF Code)		
	1 (Fop: 1)	5 (Fop: 5)	6 (Fop: 6)
Little Marvel, M410, Mini	S	S	S
New Era, Mini 93, Dark Skin Perfection, Vantage	R	S	S
Sundance II, WSU 23	R	R	S
Grant, New Season	R	S	R
WSU 28, WSU 31, 74SN5	R	R	R

7.	Establishment pathogenicity	Test on susceptible plants
8.	Multiplication inoculum	
8.1	Multiplication medium	Multiplication on agar medium: malt Agar or PDA for example
8.4	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.
8.6	Harvest of inoculum	see 10.1
8.7	Check of harvested inoculum	see 10.2
8.8	Shelflife/viability inoculum	between 4 and 8 hours, keep cool to prevent germination of spores. Viability of spores kept at -20 should be more than 3 years if stored at -20°C.
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	Race 1: Susceptible controls: Bartavelle Resistant controls: New Era and Nina Race 5: Susceptible controls: Little Marvel, Mini Resistant controls: Sundance II Race 6: Susceptible controls: Little Marvel, Mini Resistant controls: Grant
9.5	Test facility	Climate room or greenhouse.
9.6	Temperature	20-25°C
9.7	Light	12 hours or longer

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9.9	Special measures	It is important to compare the inoculated plants with the negative non inoculated control plants of the same sample. This allows interpretation of symptoms of root rot, senescence or 'wilting' caused by the stress of having roots cutted and not caused by *F. oxysporum* infection.
10.	Inoculation	
10.1	Preparation inoculum	For agar plates, remove hyphen fragments by filtering solution through muslin. For liquid medium, 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), 750 ml 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 the apical 2/3 of the roots with scissors , dip the root of the seedling in the spores suspension for 1 to 5 minutes and transplant in clean 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	Class 0: no symptoms or equivalent to negative control, 1 or 2 wilted/dried lower leaves and slight reduction in growth compared to negative control of same variety are acceptable Class 1: Range from a few chlorotic or wilted/dried leaves not present on, or more than on the negative control, up to many leaves with symptoms of senescence or wilting, some leaf drop, upper part of the plant still green and growing Class 2: Range from most of the plant wilted/dried but still alive, to plants brown and dead with stem collapsed. Classes 0 and 1 are resistant. Class 2 is susceptible

Class 0
resistant

Class 1
resistant

Class 2
susceptible

		Remark for Race 1: 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. Remark for Race 5 and 6: (Are there special remarks?)
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.

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