UPOV

International Union for the Protection of New Varieties of Plants

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PARTIAL REVISION OF THE TEST GUIDELINES FOR SPINACH

Document prepared by an expert from the Netherlands

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1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for spinach (document TG/55/7) Rev. 4.

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 Test Guidelines for Spinach (document TG/55/7 Rev. 4) be partially revised for Characteristics 17 and 18 (see document TWV/51/16 "Report", Annex IV).

3. The following changes are proposed:

(a) Revision of Characteristic 17 "Seed: spines (harvested seed)":
  (i) Addition of a state of expression with note 2 "partially present" and changing note 9 to note 3 and to reword state of expression "fully present";
  (ii) Revision of the explanation Ad. 17 in Chapter 8.2 "Explanations for individual characteristics".

(b) Revision of Characteristic 18 "Resistance to *Peronospora farinosa* f. sp. *spinaciae*":
  (i) Addition of "Race Pfs: 17", including example varieties and to add to TQ 7.3;
  (ii) Revision of explanation Ad. 18 in Chapter 8.2 "Explanations for individual characteristics".
  (iii) Addition of "Race Pfs: 17" to Chapter TQ 7.3 "Other information"

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

5. The changes in yellow highlight and underline (insertion) and ~~strikethrough~~ (deletion) had been agreed by the TWV at its fifty-first session held in Roelofarendsveen, Netherlands, July 3 to 7, 2017, and have been circulated to the Technical Committee for adoption by correspondence with a deadline of September 20, 2018 (see Circular E-18/091).

Proposal to revise Characteristic 17 “Seed: spines (harvested seed)”

Current wording

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
17. **(+)**	**VG**	**Seed: spines (harvested seed)**	**Semence : épines (grains récoltés)**	**Samen: Stacheln (geernteter Samen)**	**Semilla: espinas (semilla cosechada)**		
QL		absent	absentes	fehlend	ausentes	Resistoflay	1
		present	présentes	vorhanden	presentes	Breedblad Scherpzaad, Marimba	9

Proposed new wording

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
17. **(+)**	**VG**	**Seed: spines (harvested seed)**	**Semence : épines (grains récoltés)**	**Samen: Stacheln (geernteter Samen)**	**Semilla: espinas (semilla cosechada)**		
QL		absent	absentes	fehlend	ausentes	Resistoflay	1
		partially present	partiellement présentes	teilweise vorhanden	parcialmente presentes	Benten, Quinto	2
		fully present	pleinement présentes	vollständig vorhanden	completamente presentes	Breedblad Scherpzaad, Marimba	~~9~~ 3

Current explanation

Ad. 17: Seed: spines (harvested seed)

1
absent

9
present

Proposed new explanation

Ad. 17: Seed: spines (harvested seed)

Observations should be made on female and monoecious plants when the seed is fully grown. A plant has either only seeds without spines (round seeds), or only seeds with spines (spined seeds): A plant has only one type of seeds. Varieties may consist of only round seeded plants (1: spines absent) , round seeded plants as well as spine seeded plants (2: spines partially present) or only spine seeded plants (3: spines present).

~~1~~
spines absent

~~9~~
spines present

Proposed change to Characteristic 18 “Resistance to *Peronospora farinosa* f. sp. *spinaciae*”

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
18. **(+)**	**VG**	**Resistance to *Peronospora farinosa* f. sp. *spinaciae* (Pfs)**	**Résistance à *Peronospora farinosa* f. sp. *spinaciae* (Pfs)**	**Resistenz gegen *Peronospora farinosa* f. sp. *spinaciae* (Pfs)**	**Resistencia a *Peronospora farinosa* f. sp. *spinaciae* (Pfs)**		
		[18.1 to 18.10]	[18.1 à 18.10]	[18.1 bis 18.10]	[18.1 a 18.10]		
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18.11		**Race Pfs: 12**	**Race Pfs: 12**	**Pathotyp Pfs: 12**	**Raza Pfs: 12**		
QL		absent	absente	fehlend	ausente	Boeing, Campania	1
		present	présente	vorhanden	presente	Finch, Pigeon, Red Kitten, Zebu	9
		---	---	---	---	---	---
18.12		**Race Pfs: 13**	**Race Pfs: 13**	**Pathotyp Pfs: 13**	**Raza Pfs: 13**		
QL		absent	absente	fehlend	ausente	Campania	1
		present	présente	vorhanden	presente	Boeing, Lion	9
		---	---	---	---	---	---
18.13		**Race Pfs: 14**	**Race Pfs: 14**	**Pathotyp Pfs: 14**	**Raza Pfs: 14**		
QL		absent	absente	fehlend	ausente	Campania, Pigeon	1
		present	présente	vorhanden	presente	Califlay, Lion	9
		---	---	---	---	---	---
18.14		**Race Pfs: 15**	**Race Pfs: 15**	**Pathotyp Pfs: 15**	**Raza Pfs: 15**		
QL		absent	absente	fehlend	ausente	Caladonia	1
		present	présente	vorhanden	presente	Pigeon	9
		---	---	---	---	---	---
18.15		**Race Pfs: 16**	**Race Pfs: 16**	**Pathotyp Pfs: 16**	**Raza Pfs: 16**		
QL		absent	absente	fehlend	ausente	Meerkat	1
		present	présente	vorhanden	presente	Caladonia	9
		---	---	---	---	---	---
18.16		**Race Pfs: 17**	**Race Pfs: 17**	**Pathotyp Pfs: 17**	**Raza Pfs: 17**		
QL		absent	absente	fehlend	ausente	Pigeon	1
		present	présente	vorhanden	presente	Hydrus	9

Current explanation

Ad. 18: Resistance to *Peronospora farinosa* f. sp. *spinaciae*

Maintenance of races

Type of medium:	Living host plants, obtainable from: Naktuinbouw P.O. Box 40 NL-2370 AA Roelofarendsveen Netherlands www.naktuinbouw.com or plant material with spores stored at -20° C for a maximum of one year

Execution of test

Growth stage of plants:	First cotyledons/leaf, eleven-day-old plants
Temperature:	15°C during day/12°C during night
Light:	15 hours per day, after emergence
Growing method:	In soil in pots or trays in a glasshouse or growth chamber
Method of inoculation:	Sporulating leaves, taken from host plants that were infected seven days before, are thoroughly rinsed with sterile tap water (maximum 150 ml water per 224 plants). The spore suspension is filtered through cheesecloth and sprayed on test plants until the inoculum covers the leaves but does not run off. 150 ml of suspension is enough for up to 3 x 224 plants. Spore density should be 20,000 to 100,000 conidia/ml water. The spore suspension should be used fresh.
Remarks:	Spinach downy mildew is wind-borne. Sporulating plants should be kept in closed containers or isolated chambers to prevent any cross-contamination. Resistant controls are needed in each multiplication and in each test to ensure the race identity. Light and humidity conditions during seedling development and incubation are critical. Optimal humidity of approximately 80-90% RH allows plant growth and fungal growth; strong light inhibits spore germination and infection. The test should be carried out in wintertime with protection against direct sunshine. After inoculation, the plants should remain under plastic for three days. After this time, the plastic should be slightly raised during the daytime.

Duration of test

- Multiplication	harvest spores 7 days after inoculation
- Sowing to inoculation:	11 days
- Inoculation to reading:	10 days
Number of plants tested	56 plants
Evaluation of infection:	Resistance is usually complete; sometimes necrotic spots are visible as a result of infection. Susceptible plants show varying degrees of sporulation. Sporulation is visible as a grey covering on leaves, starting on the more humid abaxial side.

Differential varieties to identify races

Races Pfs: 1-8 and 10-15 of *Peronospora farinosa* f. sp. *spinaciae* are defined with a standard set of "differential varieties" according to the following table:

Differential variety	Pfs: 1	Pfs: 2	Pfs: 3	Pfs: 4	Pfs: 5	Pfs: 6	Pfs: 7	Pfs: 8	Pfs: 10	Pfs: 11	Pfs: 12	Pfs: 13	Pfs:14	Pfs: 15
Viroflay	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Resistoflay	R	R	S	S	S	S	S	S	S	S	S	S	S	S
Califlay	R	S	R	S	R	S	S	R	S	R	R	S	R	S
Clermont	R	R	R	R	S	S	S	S	S	S	S	S	S	R
Campania	R	R	R	R	R	S	R	S	S	R	S	S	S	R
Boeing	R	R	R	R	R	R	R	S	S	R	S	R	S	R
Lion	R	R	R	R	R	R	R	R	S	R	R	R	R	R
Lazio	R	R	R	R	R	R	R	R	R	S	S	S	S	R
Whale	R	R	R	R	R	R	R	R	S	R	R	S	R	S
Pigeon	R	R	R	R	R	R	R	R	R	R	R	R	S	R
Caladonia	R	R	R	R	R	R	R	R	R	R	R	R	R	S

Legend: R= resistance present; S = resistance absent, susceptible

Proposed new explanation

Maintenance of races

Type of medium:	Living host plants, obtainable from: Naktuinbouw P.O. Box 40 NL-2370 AA Roelofarendsveen Netherlands www.naktuinbouw.com or plant material with spores stored at -20° C for a maximum of one year

Execution of test

Growth stage of plants:	First cotyledons/leaf, eleven-day-old plants
Temperature:	15°C during day/12°C during night
Light:	15 hours per day, after emergence
Growing method:	In soil in pots or trays in a glasshouse or growth chamber
Method of inoculation:	Sporulating leaves, taken from host plants that were infected seven days before, are thoroughly rinsed with sterile tap water (maximum 150 ml water per 224 plants). The spore suspension is filtered through cheesecloth and sprayed on test plants until the inoculum covers the leaves but does not run off. 150 ml of suspension is enough for up to 3 x 224 plants. Spore density should be 20,000 to 100,000 conidia/ml water. The spore suspension should be used fresh.
Remarks:	Spinach downy mildew is wind-borne. Sporulating plants should be kept in closed containers or isolated chambers to prevent any cross-contamination. Resistant controls are needed in each multiplication and in each test to ensure the race identity. Light and humidity conditions during seedling development and incubation are critical. Optimal humidity of approximately 80-90% RH allows plant growth and fungal growth; strong light inhibits spore germination and infection. The test should be carried out in wintertime with protection against direct sunshine. After inoculation, the plants should remain under plastic for three days. After this time, the plastic should be slightly raised during the daytime.

Duration of test

- Multiplication	harvest spores 7 days after inoculation
- Sowing to inoculation:	11 days
- Inoculation to reading:	10 days

Number of plants tested	56 plants at least 20 plants

Evaluation of infection:

Resistance is usually complete; sometimes necrotic spots are visible as a result of infection. Some varieties may have a slightly lower level of resistance. In the table this is indicated by (-), showing for example a slight tip sporulation.
Susceptible plants show varying degrees of sporulation. Sporulation is visible as a grey covering on leaves, starting on the more humid abaxial side.

S: Susceptible

(R): Resistant, but slightly lower level

~~Differential varieties to identify races~~
Disease resistance reactions of spinach downy mildew races on IWGP differentials.

Races Pfs: 1-8 and 10-~~15~~ 17 of *Peronospora farinosa* f. sp. *spinaciae* are defined with a standard set of "~~differential varieties"~~ differentials according to the following table:

Differentials and type isolates are available at Naktuinbouw:

Naktuinbouw
P.O. Box 40
NL-2370 AA Roelofarendsveen
Netherlands
www.naktuinbouw.com

Race - Pfs:																
Differential variety/NIL	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17
Viroflay	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NIL 5	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NIL 3	R	S	R	S	R	S	S	R	S	R	R	S	R	S	R	S
NIL 4	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S
NIL 6	R	S	R	R	R	S	R	S	S	R	S	(R)	S	R	R	S
NIL 1	R	R	R	R	R	R	R	S	S	R	S	R	S	R	R	S
Whale	R	R	R	(R)	R	(R)	(R)	R	(R)	R	R	S	R	(R)	R	S
Pigeon	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S
Caladonia	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S
Meerkat	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	(R)
Hydrus	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Legend: S = susceptible, R or (R) = resistant

http://www.worldseed.org/wp-content/uploads/2018/04/Spinach-downy-mildew_April2018.pdf

Proposal to add “Race Pfs: 16” to TQ 7.3 “Other information”

“7.3 Other information

[…]

“(b) Resistance to pests and diseases (specify)

“(i) Resistance to *Peronospora farinosa* f. sp. *spinaciae*

Race Pfs: 1	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 2	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 3	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 4	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 5	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 6	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 7	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 8	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 10	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 11	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 12	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 13	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 14	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 15	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 16	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 17	[ ] absent	[ ] present	[ ] not tested

[…]”

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