

UPOV

TG/55/7(proj.1)

ORIGINAL: English

DATE: 2006-05-18

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

SPINACH

UPOV Code: SPINA_OLE

Spinacea oleracea L.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by an expert from the Netherlands

*to be considered by the Technical Working Party for Vegetables (TWV)
at its fortieth session to be held in Guanajuato, Guanajuato State, Mexico,
from June 12 to 16, 2006*

Alternative Names: *

<i>Botanical name</i>	<i>English</i>	<i>French</i>	<i>German</i>	<i>Spanish</i>
<i>Spinacea oleracea</i> L.	Spinach			

The purpose of these guidelines ("Test Guidelines") is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These Test Guidelines should be read in conjunction with the General Introduction and its associated TGP documents.

* These names were correct at the time of the introduction of these Test Guidelines but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Website (www.upov.int), for the latest information.]

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Spinacea oleracea* L.

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must make sure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of seed.

2.3 The minimum quantity of plant material or seed to be supplied by the applicant, should be:

20,000 seeds

The seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority.

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 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.

3. Method of Examination

3.1 *Number of Growing Cycles*

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

3.2 *Testing Place*

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

3.3 *Conditions for Conducting the Examination*

3.3.1 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.

3.3.2 The recommended method of observing the characteristic is indicated by the following key in the second column of the Table 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”

3.4 *Test Design*

3.4.1 Each test should be designed to result in a total of 200 drilled plants and/or 100 single spaced plants which should be divided between 2 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 *Number of Plants / Parts of Plants to be Examined*

Unless otherwise indicated, all observations should be made on 60 plants or parts taken from each of 60 plants.

3.6 *Additional Tests*

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 *Distinctness*

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

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 Test Guidelines are familiar with the

recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.2 *Uniformity*

4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

(a) *Seed-propagated open-pollinated varieties*

The assessment of uniformity of seed-propagated open-pollinated varieties should be according to the recommendations for cross-pollinated varieties in the General Introduction.

(b) *F1 hybrids*

For the assessment of uniformity of F1 hybrid varieties, a population standard of 2 % and an acceptance probability of at least 95 % should be applied. This excludes clearly recognizable inbred plants. In the case of such clearly recognizable inbred plants an additional assessment may be made with a population standard of 3 % and an acceptance probability of at least 95 %. In the case of a sample size of 200 drilled plants, 7 off-types are allowed with an additional 10 clearly recognizable inbred plants. In the case of a sample size of 100 single spaced plants, 5 off-types are allowed with an additional 6 clearly recognizable inbred plants.

4.3 *Stability*

4.3.1 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.

4.3.2 Where appropriate, or in cases of doubt, stability may be tested, either by growing a further generation, or by testing a new seed or plant stock to ensure that it exhibits the same characteristics as those shown by the previous material supplied.

5. Grouping of Varieties and Organization of the Growing Trial

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

- (a) Seed: Spines (characteristic 1)
- (b) Flowering plants: proportion of monoecious plants (characteristic 13)
- (c) Flowering plants: proportion of male plants (characteristic 15)
- (d) Start of bolting (for spring sown crop, 15 % of plants) (characteristic 16)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction.

6. Introduction to the Table of Characteristics

6.1 *Categories of Characteristics*

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

6.2 *States of Expression and Corresponding Notes*

States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

6.3 *Types of Expression*

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 *Example Varieties*

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

6.5 *Legend*

(*) Asterisked characteristic – see Chapter 6.1.2

QL: Qualitative characteristic – see Chapter 6.3

QN: Quantitative characteristic – see Chapter 6.3

PQ: Pseudo-qualitative characteristic – see Chapter 6.3

MG: single measurement of a group of plants or parts of plants – see Chapter 3.3.1

MS: measurement of a number of individual plants or parts of plants – see Chapter 3.3.1

VG: visual assessment by a single observation of a group of plants or parts of plants – Chapter 3.3.1

VS: visual assessment by observation of individual plants or parts of plants” –see Chapter 3.3.1

(a)-(c) See Explanations on the Table of Characteristics in Chapter 8.1

(+) See Explanations on the Table of Characteristics in Chapter 8.2

7. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

	English	français	deutsch	español	Example Varieties Exemples Beispielsorten Variedades ejemplo	Note/ Nota
1. (*)	VG Seed: spines	Semence: épines	Samen: Stacheln	Semilla: espinas		
QL	absent	absentes	fehlend	ausentes	Butterflay	1
	present	présentes	vorhanden	presentes	Bergola, Subito	9
2.	VG Seedling: length of cotyledon	Plantule: longueur du cotylédon	Sämling: Länge des Keimblatts	Plántula: longitud del cotiledón		
QN	short	court	kurz	corto	Beta, Nores	3
	medium	moyen	mittel	medio		5
	long	long	lang	largo	Breedblad Scherpzaad, Resistoflay, Subito	7
3. (*)	VG Leaf blade: intensity of green color	Limbe: intensité de la couleur verte	Blattspreite: Intensität der Grünfärbung	Limbo: intensidad del color verde		
QN (a)	very light	très claire	sehr hell	muy claro	Virtuosa	1
	light	claire	hell	claro	Subito	3
	medium	moyenne	mittel	medio	Butterflay, Monnopa	5
	dark	foncée	dunkel	oscuro	Lavewa, Trinidad, Wobli	7
	very dark	très foncée	sehr dunkel	muy oscuro	Lorelay	9
4. (*)	VG Leaf blade: blistering	Limbe: cloûre	Blattspreite: Blasigkeit	Limbo: abullonado		
QN (a)	absent or very weak	absente ou très faible	fehlend oder gering	sehr ausente o muy débil		1
	weak	faible	gering	débil	Polka, Prince, Vital	3
	medium	moyenne	mittel	medio	Beta, Butterflay	5
	strong	forte	stark	fuerte	Martine, Rhythm	7
	very strong	très forte	sehr stark	muy fuerte	Bloomsdale Longstanding	9

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
5 (*)	VG Leaf blade: lobing	Limbe: découpure du bord	Blattspreite: Lappung	Limbo: lobulado		
QN (a)	absent or very weak	absente ou très faible	fehlend oder gering	sehr ausente o muy débil		1
	weak	faible	gering	débil	Butterflay	3
	medium	moyenne	mittel	medio		5
	strong	forte	stark	fuerte	Tamara	7
	very strong	très forte	sehr stark	muy fuerte	Münsterländer	9
6. (*) (+)	VG Petiole: attitude	Pétiole: port	Blattstiel: Haltung	Peciolo: porte		
QN (a)	erect	dressé	aufrecht	erecto	Bloomsdale Longstanding	1
	semi-erect	demi-dressé	halbaufrecht	semi-erecto	Monnopa, Prince, Subito	3
	horizontal	horizontal	waagrecht	horizontal	Comte, Lavewa	5
7.	VG Petiole: length	Pétiole: longueur	Blattstiel: Länge	Peciolo: longitud		
QN (a)	short	court	kurz	corto		3
	medium	moyen	mittel	medio	Butterflay	5
	long	long	lang	largo	Resistoflay	7
8. (*) (+)	VG Leaf blade: attitude	Limbe: port	Blattspreite: Haltung	Limbo: porte		
QN (a)	erect	dressé	aufrecht	erecto		1
	semi-erect	demi-dressé	halbaufrecht	semi-erecto	Monnopa, Prince, Subito	3
	horizontal	horizontal	waagrecht	horizontal	Comte, Lavewa	5
	semi-pendulous	demi-retombant	halbhängend	semi-colgante	Medania	7

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
9. (*)	VG	Leaf blade: shape (excluding basal lobes)	Limbe: forme (à l'exclusion des lobes basals)	Blattspreite: Form (Basallappen ausgenommen)	Limbo: forma (excluyendo lóbulos basales)	
PQ	(a)	elliptic	elliptique	elliptisch	elíptica	1
		broad elliptic	elliptique large	breit elliptisch	elíptica ancha	Ass, Comte, Nores
		circular	circulaire	rund	circular	3
		ovate	ovale	eiförmig	oval	Lavewa, Prince, Resistoflay
		broad ovate	ovale large	breit eiförmig	oval ancha	Butterflay
		triangular	triangulaire	dreieckig	triangular	Maracas
10.	VG	Leaf blade: curving of margin	Limbe: courbure du bord	Blattspreite: Biegung des Randes	Limbo: curvado del margen	
QN	(a)	incurved	incurvé	eingebogen	incurvado	Estivato
		flat	plan	flach	plano	Resistoflay
		recurved	récurvé	umgebogen	recurvado	Ass
11. (*)	VG	Leaf blade: shape of apex	Limbe: forme de la pointe	Blattspreite: Form der Spitze	Limbo: forma del ápice	
QN	(a)	acute	aigüe	spitz	agudo	Rhythm
		obtuse	obtuse	stumpf	obtuso	Prince, Resistoflay, Subito
		rounded	arrondie	abgerundet	redondeado	Ass, Comte
12. (*)	VG	Leaf blade: shape in longitudinal section	Limbe: forme en section longitudinale	Blattspreite: Form im Längsschnitt	Limbo: forma en sección longitudinal	
QN	(a)	concave	concave	konkav	cóncava	1
		flat	plat	flach	plana	Resistoflay
		convex	convexe	konvex	convexa	Ass

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
13. (*)(+)	VG Flowering plants: proportion of monoecious plants	Plantes en floraison: proportion de plantes monoïques	Blühende Pflanzen: Anteil monözischer Pflanzen	Plantas en floración: proporción de plantas monoicas		
QN (b)	absent or very low	absente ou très faible	fehlend oder sehr gering	ausente o muy baja	Ass, Medania	1
	low	faible	gering	baja	Comte, Matador	3
	medium	moyenne	mittel	media	Spencer	5
	high	grande	hoch	alta	Beta	7
	very high	très grande	sehr hoch	muy alta	Monnopa, Trinidad	9
14. (*)(+)	VG Flowering plants: proportion of female plants	Plantes en floraison: proportion de plantes femelles	Blühende Pflanzen: Anteil weiblicher Pflanzen	Plantas en floración: proporción de plantas femeninas		
QN (b)	absent or very low	absente ou très faible	fehlend oder sehr gering	ausente o muy baja	Monnopa, Trinidad	1
	low	faible	gering	baja	Beta, Comte	3
	medium	moyenne	mittel	media	Medania, Spencer	5
	high	grande	hoch	alta		7
	very high	très grande	sehr hoch	muy alta		9
15. (*)(+)	VG Flowering plants: proportion of male plants	Plantes en floraison: proportion de plantes mâles	Blühende Pflanzen: Anteil männlicher Pflanzen	Plantas en floración: proporción de plantas masculinas		
QN (b)	absent or very low	absente ou très faible	fehlend oder sehr gering	ausente o muy baja	Beta, Monnopa, Trinidad	1
	low	faible	gering	baja		3
	medium	moyenne	mittel	media	Ass, Comte, Medania	5
	high	grande	hoch	alta		7
	very high	très grande	sehr hoch	muy alta		9

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
16. (*)	VG Start of bolting (for spring sown crop, 15% of plants)	Début de montaison (pour des variétés semées au printemps, 15% de plantes)	Schoßbeginn (bei Frühjahrssaussaat, 15% der Pflanzen)	Comienzo del espigado (para cultivos sembrados en primavera, 15% de las plantas)		
QN (b)	very early	très précoce	sehr früh	muy temprano	Maracas	1
	early	précoce	früh	temprano	Subito	3
	medium	moyen	mittel	medio	Monnopa	5
	late	tardif	spät	tardío	Medania, Wobli	7
	very late	très tardif	sehr spät	muy tardío	Chica, Lavewa, Spencer	9
17. (+)	VG Resistance to <u>Peronospora farinosa</u> f. <u>spinaciae</u>	Résistance à <u>Peronospora farinosa</u> f. <u>spinaciae</u>	Resistenz gegen <u>Peronospora farinosa</u> f. <u>spinaciae</u>	Resistencia a <u>Peronospora farinosa</u> f. <u>spinaciae</u>		
QL	-----	-----	-----	-----	-----	-----
17.1	Race Pfs:1	Race Pfs:1	Pathotyp Pfs:1	Raza Pfs:1		
	absent	absente	fehlend	ausente	Viroflay, Winterreuzen	1
	present	présente	vorhanden	presente	Califlay, Resistoflay	9
	-----	-----	-----	-----	-----	-----
					-	
17.2	Race 2	Race 2	Pathotyp 2	Raza Pfs:2		
	absent	absente	fehlend	ausente	Califlay	1
	present	présente	vorhanden	presente	Resistoflay	9
	-----	-----	-----	-----	-----	-----
					-	
17.3	Race Pfs: 3	Race Pfs: 3	Pathotyp Pfs:3	Raza Pfs:3		
	absent	absente	fehlend	ausente	Resistoflay	1
	present	présente	vorhanden	presente	Clermont, Califlay	9
	-----	-----	-----	-----	-----	-----
					-	

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
17.4	Race Pfs: 4	Race Pfs: 4	Pathotyp Pfs:4	Raza Pfs:4		
	absent	absente	fehlend	ausente	Califlay	1
	present	présente	vorhanden	presente	Clermont	9
					-	
17.5	Race Pfs: 5	Race Pfs: 3	Pathotyp Pfs:3	Raza Pfs:5		
	absent	absente	fehlend	ausente	Clermont	1
	present	présente	vorhanden	presente	Campania, Califlay	9
					-	
17.6	Race Pfs: 6	Race Pfs: 3	Pathotyp Pfs:3	Raza Pfs:6		
	absent	absente	fehlend	ausente	Campania, Califlay	1
	present	présente	vorhanden	presente	Boeing	9
					-	
17.7	Race Pfs: 7	Race Pfs: 3	Pathotyp Pfs:3	Raza Pfs:7		
	absent	absente	fehlend	ausente	Califlay	1
	present	présente	vorhanden	presente	Campania	9
					-	
17.8	Race Pfs: 8	Race Pfs: 3	Pathotyp Pfs:3	Raza Pfs:8		
	absent	absente	fehlend	ausente	Boeing, Campania	1
	present	présente	vorhanden	presente	Lazio, Lion	9
					-	
17.9	Race Pfs: 9	Race Pfs: 3	Pathotyp Pfs:3	Raza Pfs:9		
	absent	absente	fehlend	ausente	Campania	1
	present	présente	vorhanden	presente	Lazio, Lion, Boeing	9
					-	

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
17.10	Race Pfs: 10	Race Pfs: 10	Pathotyp Pfs:10	Raza Pfs:10		
	absent	absente	fehlend	ausente	Lion, Boeing, Campania	1
	present	présente	vorhanden	presente	Lazio	9
18.	VG Resistance to Cucumber mosaic virus (CMV)	Résistance au virus de la mosaïque du concombre (CMV)	Resistenz gegen Gurkenmosaikvirus (CMV)	Resistencia al virus del mosaico del pepino (CMV)		
(+)						
QL	absent	absente	fehlend	ausente	Polka	1
	present	présente	vorhanden	presente	Symphony	9

8. Explanations on the Table of Characteristics

8.1 Explanations covering several characteristics

Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

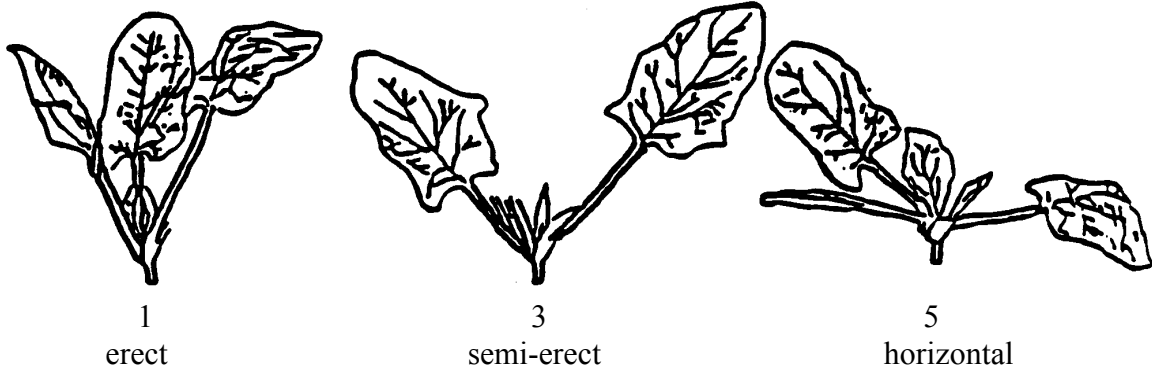
- (a) observations on the leaf blade should be made on the seventh to tenth leaves of the adult not bolted plant. The shape of the leaf blade in longitudinal section should be observed on central leaves.
- (b) observations on the proportion of monoecious, female or male plants (characteristics 13 to 15) should be made at the beginning of seed setting. The three groups are defined as follows:

Monoecious plants: plants which have both male and female flowers with seeds clearly visible

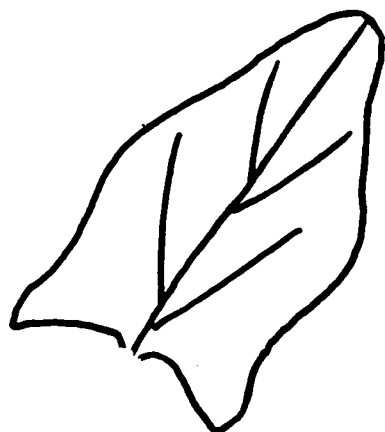
Female plants: plants which have only female flowers with seeds clearly visible

Male plants: plants which have only male flowers.

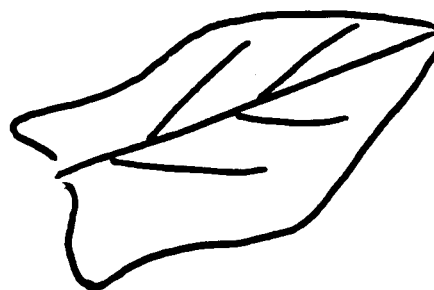
Ad. 6: Petiole: attitude



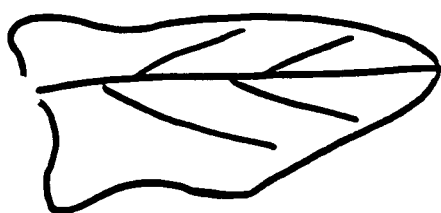
Ad. 8: Leaf blade: attitude



1
erect



3
semi-erect



5
horizontal



7
semi-pendulous

Ad. 13 + 14 + 15: Flowering plants: proportion of monoecious (14)/female (15)/male (16) plants

	<u>Note</u>	<u>Approximate percentage</u>
absent or very low	1	< 10 %
low	3	30 %
medium	5	50 %
high	7	70 %
very high	9	> 90 %

Ad. 17: Resistance to *Peronospora farinosa* f. sp. *spinaciae*

Maintenance of races

Type of medium: Living host plants, obtainable from Naktuinbouw, Roelofarendsveen, Netherlands; or plant material with spores stored at -20° C for max.. 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 cheese cloth and sprayed on test plants till inoculum covers the leaves but does not run off. 150 ml of suspension is enough for up to 3 x 224 plants. Spore density is 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 ca. 80-90% RH allows plant growth and fungal growth; strong light will inhibit spore germination and infection

Test is carried out in wintertime and protected against direct sunshine. After inoculation, the plants remain for three days under plastic, after this during daytime the plastic is slightly lifted.

Duration of test:

- Multiplication harvest spores 7 d 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 cover on leaves, starting on the more humid abaxial side.

Control varieties to identify races:

Races Pfs:1-10 of *Peronospora farinosa* f. sp. *spinaciae* are defined with a standard set of so-called differential varieties according to a table published by ISF at www.worldseed.org and copied here:

Differential variety	Pfs:1	Pfs:2	Pfs:3	Pfs:4	Pfs:5	Pfs:6	Pfs:7	Pfs:8	Pfs:9	Pfs:10
Viroflay	S	S	S	S	S	S	S	S	S	S
Resistoflay	R	R	S	S	S	S	S	S	S	S
Califlay	R	S	R	S	R	S	S	R	R	S
Clermont	R	R	R	R	S	S	S	S	S	S
Campania	R	R	R	R	R	S	R	S	S	S
Boeing	R	R	R	R	R	R	R	S	R	S
Lion	R	R	R	R	R	R	R	R	R	S
Lazio	R	R	R	R	R	R	R	R	R	R

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

Ad 18: Resistance to Cucumber mosaic virus (CMV)

Maintenance and propagation of isolates

Storage of medium: on leaves in freezer or desiccated over CaCl₂

Special conditions: Isolates NL 16 and SP 43 obtainable at PRI, Wageningen, Netherlands

Propagation: on susceptible cucumber plants

Execution of test

Growth stage of plants: when two or three true leaves are present

Temperature: 20°C during the day, 18°C during the night

Light: at least 16 hours per day

Growing method: plants grown in module 5 x 5 cm (potting soil)

Preparation of inoculum: a mixture of isolates is ground in water (dilution 1:10)

Method of inoculation: plants are dusted with carborundum powder on two to three leaves and then rubbed with a sponge soaked in inoculum. After inoculation the plants are lightly rinsed with water.

Remarks: due to climatic conditions, the test is best carried out from February to June (Northern Hemisphere).

Observations

Time of observation: 7 to 9 days after inoculation

Symptoms:

resistant plant: no symptoms

sensitive plant: dwarf growth, mosaic symptoms in the heart of the plants

Differential host varieties to be used:

susceptible variety: Polka

resistant variety: Symphony

9. Literature

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Kobabe, G., 1972: Die Vererbung der männlichen Sterilität beim Spinat (*Spinacea oleracea* L.) und Möglichkeiten der Nutzung dieser Eigenschaft in der Hybridzüchtung, *Zeitschrift für Pflanzenzüchtung* 67, 233-242, Paul Parey Verlag, Berlin und Hamburg.

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Shinohara, S., 1984: *Vegetable Seed Production Technology of Japan*. Elucidated with respective variety development histories, Particulars. Vol I, 1984, SAACEO, Tokyo.

van Oorschot, J.L.P., 1960: Effects of daylength upon growth and development of spinach (*Spinacea oleracea* L.), *Meded. Landbouwhogeschool, Wageningen*, 60 (18), 1-10.

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10. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
		Application date: (not to be filled in by the applicant)
TECHNICAL QUESTIONNAIRE to be completed in connection with an application for plant breeders' rights		
1. Subject of the Technical Questionnaire		
1.1 Botanical name	<input type="text" value="Spinacea oleracea L."/>	
1.2 Common name	<input type="text" value="Spinach"/>	
2. Applicant		
Name	<input type="text"/>	
Address	<input type="text"/>	
Telephone No.	<input type="text"/>	
Fax No.	<input type="text"/>	
E-mail address	<input type="text"/>	
Breeder (if different from applicant)	<input type="text"/>	
3. Proposed denomination and breeder's reference		
Proposed denomination (if available)	<input type="text"/>	
Breeder's reference	<input type="text"/>	

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
<p>#4. Information on the breeding scheme and propagation of the variety</p> <p>4.1 Breeding scheme</p> <p>(a) Hybrid <input type="checkbox"/> [] (please state parent varieties)</p> <p>(b) open-pollinated variety <input type="checkbox"/> [] (please state known parent variety(ies))</p> <p>(d) Other <input type="checkbox"/> [] (please provide details)</p>		

Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:	
<p>5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).</p>			
Characteristics	Example Varieties	Note	
<p>5.1 Seed: spines (1)</p>			
absent	Butterflay	1[]	
present	Bergola, Subito	9[]	
<p>5.2 Leaf blade: intensity of green color (3)</p>			
very light	Virtuosa	1[]	
light	Subito	3[]	
medium	Butterflay, Monnopa	5[]	
dark	Larewa, Trinidad, Wobli	7[]	
very dark	Lorelay	9[]	
<p>5.3 Leaf blade: blistering (4)</p>			
absent or very weak		1[]	
weak	Polka, Prince, Vital	3[]	
medium	Beta, Butterflay	5[]	
strong	Martine, Rhythm	7[]	
very strong	Bloomsdale Longstanding	9[]	
<p>5.4 Leaf blade: shape of apex (11)</p>			
acute	Rhythm	1[]	
obtuse	Prince, Resistoflay, Subito	2[]	
rounded	Ass, Comte	3[]	

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
Characteristics		Example Varieties	Note
5.5 Flowering plants: proportion of monoecious plants (13)			
absent or very low		Ass, Medania	1[]
low		Comte, Matador	3[]
medium		Spencer	5[]
high		Beta	7[]
very high		Monnopa, Trinidad	9[]
5.6 Flowering plants: proportion of female plants (14)			
absent or very low		Monnopa, Trinidad	1[]
low		Beta, Comte	3[]
medium		Medania, Spencer	5[]
high			7[]
very high			9[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:	
<p>6. Similar varieties and differences from these varieties</p> <p><i>Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.</i></p>			
Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the similar variety(ies)	Describe the expression of the characteristic(s) for your candidate variety
GN 33 <i>Example</i>	<i>[e.g. Flower color]</i>	<i>[e.g. orange]</i>	<i>[e.g. orange red]</i>
<p>Comments:</p>			

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:		
7. Additional information which may help to distinguish the variety				
7.1 Resistance to pests and diseases				
		absent	present	not tested
(a) <i>Peronospora farinosa</i> f. sp. <i>spinacea</i>				
Pfs:1 (characteristic 17.1)		[]	[]	[]
Pfs:2 (characteristic 17.2)		[]	[]	[]
Pfs:3 (characteristic 17.3)		[]	[]	[]
Pfs:4 (characteristic 17.4)		[]	[]	[]
Pfs:5 (characteristic 17.5)		[]	[]	[]
Pfs:6 (characteristic 17.6)		[]	[]	[]
Pfs:7 (characteristic 17.7)		[]	[]	[]
Pfs:8 (characteristic 17.8)		[]	[]	[]
Pfs:9 (characteristic 17.9)		[]	[]	[]
Pfs:10 (characteristic 17.10)		[]	[]	[]
(b) Cucumber mosaic virus (characteristic 23)		[]	[]	[]
(c) Other resistances (specify)		[]	[]	[]
.....				
7.2 Special conditions for the examination of the variety				
(a) Use:				
	- only in glasshouse		[]	
	- only in the open		[]	
	- in the open <u>and</u> in glasshouse		[]	
(a) Other conditions				
.....				
7.3 Other information				

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
<p>8. Authorization for release</p> <p>(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?</p> <p>Yes [] No []</p> <p>(b) Has such authorization been obtained?</p> <p>Yes [] No []</p> <p>If the answer to (b) is yes, please attach a copy of the authorization.</p>		

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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9. Information on plant material to be examined or submitted for examination.

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

- | | | |
|---|---------|--------|
| (a) Microorganisms (e.g. virus, bacteria, phytoplasma) | Yes [] | No [] |
| (b) Chemical treatment (e.g. growth retardant, pesticide) | Yes [] | No [] |
| (c) Tissue culture | Yes [] | No [] |
| (d) Other factors | Yes [] | No [] |

Please provide details for where you have indicated “yes”.

.....

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

Yes []

(please provide details as specified by the Authority)

No []”

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant's name

Signature

Date