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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

SPINACH

UPOV Code: SPINA_OLE

Spinacea oleracea L.

[*]

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

Alternative Names:[*]

Botanical name	*English*	*French*	*German*	*Spanish*
Spinacea oleracea L.	Spinach	Épinard	Spinat	Espinaca

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 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 on single plants should be made on 60 plants or parts taken from each of 60 plants and any other observations made on all plants in the test.

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:

4.2.2 Cross-pollinated varieties: The assessment of uniformity of cross-pollinated varieties should be according to the recommendations for cross-pollinated varieties in the General Introduction.

4.2.3 Hybrids: For the assessment of uniformity of hybrid varieties, a population standard of 2% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 100 plants, 5 off-types are allowed. In addition, a population standard of 3% and an acceptance probability of at least 95% should be applied for inbred plants obviously resulting from the selfing of a parent line. In the case of a sample size of 100 plants, 6 inbred plants are allowed.

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 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) Leaf blade: intensity of green color (characteristic 2)
(b) Leaf blade: blistering (characteristic 3)
(c) Proportion of monoecious plants (characteristic 12)
(d) Proportion of female plants (characteristic 13)
(e) Proportion of male plants (characteristic 14)
(f) Time of start of bolting (for spring sown crops, 15% of plants) (characteristic 15)
(g) Resistance to *Peronospora farinosa* f. sp. *spinaciae* (characteristic 17)

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, MS, VG, VS: See Chapter 3.3.2

(a) 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 Beispielssorten Variedades ejemplo	Note/ Nota
1.	**VG**	**Seedling: length of cotyledon**	**Plantule: longueur du cotylédon**	**Sämling: Länge des Keimblatts**	**Plántula: longitud del cotiledón**		
QN		short	court	kurz	corto	Nores	3
		medium	moyen	mittel	medio		5
		long	long	lang	largo	Breedblad Scherpzaad, Resistoflay	7
2. (*)	**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		1
		light	claire	hell	claro	Monet, Viroflay, Winterreuzen	3
		medium	moyenne	mittel	medio	Butterflay, Monnopa	5
		dark	foncée	dunkel	oscuro	Imola, Lavewa, Nores	7
		very dark	très foncée	sehr dunkel	muy oscuro	Lorelay, Mystic	9
3. (*)	**VG**	**Leaf blade: blistering**	**Limbe: cloqûre**	**Blattspreite: Blasigkeit**	**Limbo: abullonado**		
QN	**(a)**	absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	Matador	1
		weak	faible	gering	débil	Polka, Tarpy	3
		medium	moyenne	mittel	medio	Butterflay, Koala, Mystic	5
		strong	forte	stark	fuerte	Giraffe, Rhythm	7
		very strong	très forte	sehr stark	muy fuerte	Menorca, Revolver	9
4. (*) (+)	**VG**	**Leaf blade: lobing**	**Limbe: découpure du bord**	**Blattspreite: Lappung**	**Limbo: lobulado**		
QN	**(a)**	absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil		1
		weak	faible	gering	débil	Butterflay, Giraffe	3
		medium	moyenne	mittel	medio	Mystic	5
		strong	forte	stark	fuerte	Parrot	7

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
5. **(*)** **(+)**	**VG**	**Petiole: attitude**	**Pétiole: port**	**Blattstiel: Haltung**	**Pecíolo: porte**		
QN	**(a)**	erect	dressé	aufrecht	erecto	Grappa	1
		semi-erect	demi-dressé	halbaufrecht	semi-erecto	Monnopa, Parrot	3
		horizontal	horizontal	waagerecht	horizontal	Comte, Lavewa	5
6.	**VG**	**Petiole: length**	**Pétiole: longueur**	**Blattstiel: Länge**	**Pecíolo: longitud**		
QN	**(a)**	short	court	kurz	corto	Imola, Mystic	3
		medium	moyen	mittel	medio	Butterflay, Giraffe	5
		long	long	lang	largo	Grappa, Resistoflay	7
7. **(*)** **(+)**	**VG**	**Leaf blade: attitude**	**Limbe: port**	**Blattspreite: Haltung**	**Limbo: porte**		
QN	**(a)**	erect	dressé	aufrecht	erecto		1
		semi-erect	demi-dressé	halbaufrecht	semi-erecto	Grappa, Monnopa, Rhythm	3
		horizontal	horizontal	waagerecht	horizontal	Lavewa, Mystic	5
		semi-pendulous	demi-retombant	halbhängend	semi-colgante	Giraffe, Medania	7
8. **(*)**	**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)**	triangular	triangulaire	dreieckig	triangular	Grappa, Maracas	1
		medium ovate	ovale	eiförmig	oval	Lavewa, Resistoflay	2
		broad ovate	ovale large	breit eiförmig	oval ancha	Butterflay	3
		medium elliptic	elliptique moyen	mittel elliptisch	elíptica media		4
		broad elliptic	elliptique large	breit elliptisch	elíptica ancha	Nores	5
		circular	circulaire	rund	circular	Giraffe	6

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
9.	**VG**	**Leaf blade: curving of margin**	**Limbe: courbure du bord**	**Blattspreite: Biegung des Randes**	**Limbo: curvado del margen**		
QN	**(a)**	incurved	incurvé	eingebogen	incurvado		1
		flat	plan	flach	plano	Resistoflay	2
		recurved	récurvé	umgebogen	recurvado	Imola	3
10. (*)	**VG**	**Leaf blade: shape of apex**	**Limbe: forme de la pointe**	**Blattspreite: Form der Spitze**	**Limbo: forma del ápice**		
QN	**(a)**	acute	aigue	spitz	agudo	Grappa, Rhythm	1
		obtuse	obtuse	stumpf	obtuso	Resistoflay	2
		rounded	arrondie	abgerundet	redondeado	Imola, Nores	3
11. (*)	**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	Mystic, Resistoflay	2
		convex	convexe	konvex	convexa	Grappa, Lazio	3
12. (*) (+)	**VS**	**Proportion of monoecious plants**	**Proportion de plantes monoïques**	**Anteil monözischer Pflanzen**	**Proporción de plantas monoicas**		
QN		absent or very low	absente ou très faible	fehlend oder sehr gering	ausente o muy baja	Medania	1
		low	faible	gering	baja	Matador	3
		medium	moyenne	mittel	media	Figo	5
		high	grande	hoch	alta	Giraffe, Lazio	7
		very high	très grande	sehr hoch	muy alta	Monnopa	9

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
13. (*) (+)	**VS**	**Proportion of female plants**	**Proportion de plantes femelles**	**Anteil weiblicher Pflanzen**	**Proporción de plantas femeninas**		
QN		absent or very low	absente ou très faible	fehlend oder sehr gering	ausente o muy baja	Monnopa	1
		low	faible	gering	baja	Giraffe	3
		medium	moyenne	mittel	media	Figo, Medania	5
		high	grande	hoch	alta	Parrot	7
		very high	très grande	sehr hoch	muy alta		9
14. (*) (+)	**VS**	**Proportion of male plants**	**Proportion de plantes mâles**	**Anteil männlicher Pflanzen**	**Proporción de plantas masculinas**		
QN		absent or very low	absente ou très faible	fehlend oder sehr gering	ausente o muy baja	Monnopa, Parrot	1
		low	faible	gering	baja		3
		medium	moyenne	mittel	media	Medania	5
		high	grande	hoch	alta		7
		very high	très grande	sehr hoch	muy alta		9
15. (*) (+)	**MG**	**Time of start of bolting (for spring sown crops, 15% of plants)**	**L'époque du début de montaison (pour des variétés semées au printemps, 15% de plantes)**	**Zeitpunkt des Schoßbeginns (bei Frühjahrsaussaat, 15% der Pflanzen)**	**Momento del comienzo de la subido a flor (para cultivos sembrados en primavera, 15% de las plantas)**		
QN		very early	très précoce	sehr früh	muy temprano	Figo, Maracas	1
		early	précoce	früh	temprano	Bandola, Viroflay	3
		medium	moyen	mittel	medio	Matador, Monnopa	5
		late	tardif	spät	tardío	Grappa, Medania, Revolver	7
		very late	très tardif	sehr spät	muy tardío	Chica, Lavewa	9

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
16. **(+)**	**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
17. **(+)**	**VG**	**Resistance to *Peronospora farinosa* f. sp. *spinaciae***	**Résistance à *Peronospora farinosa* f. sp. *spinaciae***	**Resistenz gegen *Peronospora farinosa* f. sp. *spinaciae***	**Resistencia a *Peronospora farinosa* f. sp. *spinaciae***		
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 Pfs: 2**	**Race Pfs: 2**	**Pathotyp Pfs: 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	Califlay, Clermont	9
		---	---	---	---	---	---
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
		---	---	---	---	---	---

	English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
17.5	**Race Pfs: 5**	**Race Pfs: 5**	**Pathotyp Pfs: 5**	**Raza Pfs: 5**		
	absent	absente	fehlend	ausente	Clermont	1
	present	présente	vorhanden	presente	Califlay, Campania	9
17.6	**Race Pfs: 6**	**Race Pfs: 6**	**Pathotyp Pfs: 6**	**Raza Pfs: 6**		
	absent	absente	fehlend	ausente	Califlay, Campania	1
	present	présente	vorhanden	presente	Boeing	9
17.7	**Race Pfs: 7**	**Race Pfs: 7**	**Pathotyp Pfs: 7**	**Raza Pfs: 7**		
	absent	absente	fehlend	ausente	Califlay	1
	present	présente	vorhanden	presente	Campania	9
17.8	**Race Pfs: 8**	**Race Pfs: 8**	**Pathotyp Pfs: 8**	**Raza Pfs: 8**		
	absent	absente	fehlend	ausente	Boeing, Campania	1
	present	présente	vorhanden	presente	Lazio, Lion	9
17.9	**Race Pfs: 10**	**Race Pfs: 10**	**Pathotyp Pfs:10**	**Raza Pfs:10**		
	absent	absente	fehlend	ausente	Boeing, Campania, Lion	1
	present	présente	vorhanden	presente	Lazio	9
17.10	**Race Pfs: 11**	**Race Pfs: 11**	**Pathotyp Pfs: 11**	**Raza Pfs: 11**		
	absent	absente	fehlend	ausente	Lazio	1
	present	présente	vorhanden	presente	Boeing, Califlay, Campania, Lion	9

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
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

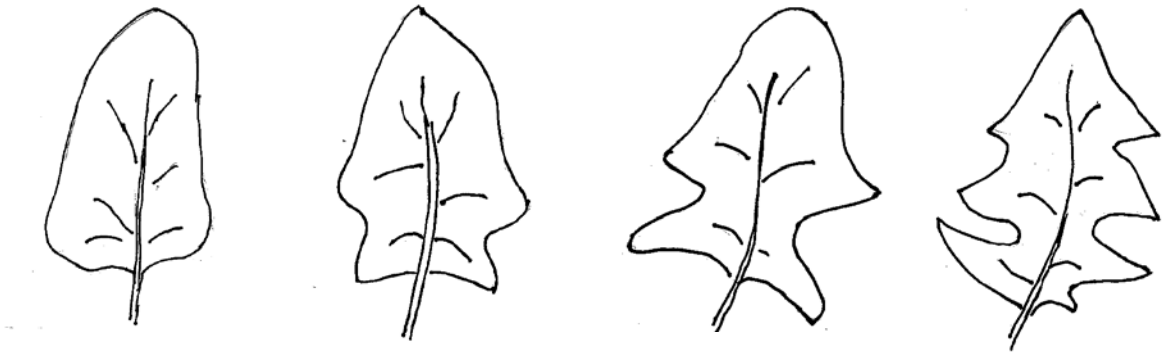
8.1 Explanations covering several characteristics

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.

8.2 Explanations for individual characteristics

Ad. 4: Leaf blade: lobing



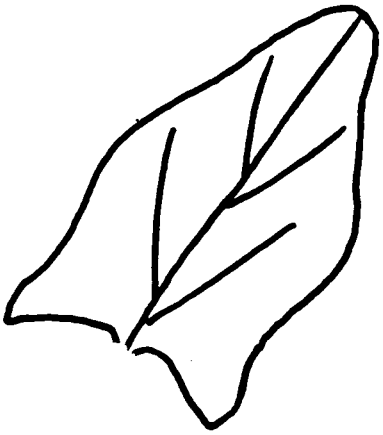
1	3	5	7
absent or very weak	weak	medium	strong

Ad. 5: Petiole: attitude

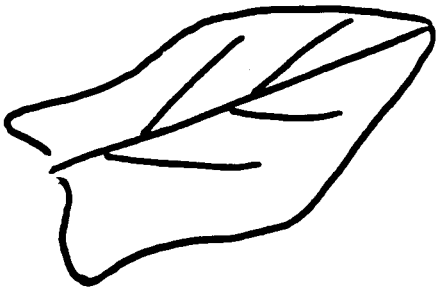
1	3	5
erect	semi-erect	horizontal

Ad. 7: Leaf blade: attitude

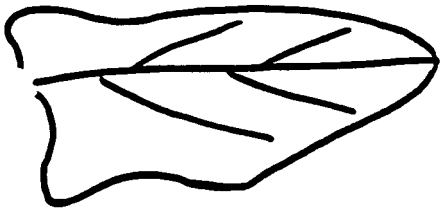
To be observed in relation to the horizontal, independent of the attitude of the petiole (characteristic 5).



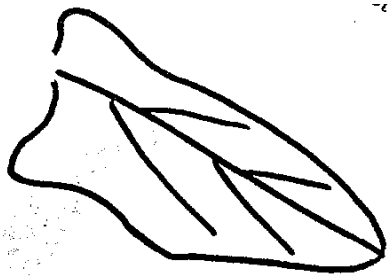
1
erect



3
semi-erect



5
horizontal



7
semi-pendulous

Ad. 12: Proportion of monoecious plants
Ad. 13: Proportion of female plants
Ad. 14: Proportion of male plants

Observations on the proportion of monoecious, female or male plants should be made at the beginning of seed setting. The three groups are defined as follows:

Monoecious plants:	plants which have both male flowers and female flowers (seeds clearly visible);
Female plants:	plants which have only female flowers (seeds clearly visible);
Male plants:	plants which have only male flowers.

	Note	Approximate percentage
absent or very low	1	< 10%
	2	20%
low	3	30%
	4	40%
medium	5	50%
	6	60%
high	7	70%
	8	80%
very high	9	> 90%

Ad. 15: Time of start of bolting (for spring sown crops, 15% of plants)

The time of bolting of a plant is when the central flowering stem appears through stretching of the internodes.

Ad. 16: Seed: spines (harvested seed)

1
absent

9
present

Ad. 17: 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 10-11 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
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	S	R
Clermont	R	R	R	R	S	S	S	S	S	S
Campania	R	R	R	R	R	S	R	S	S	R
Boeing	R	R	R	R	R	R	R	S	S	R
Lion	R	R	R	R	R	R	R	R	S	R
Lazio	R	R	R	R	R	R	R	R	R	S

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 $CaCl_2$

Special conditions: Isolates NL 16 and SP 43 which can be obtained from:
PRI (Plant Research International)
Prime Diagnostics
P.O. Box 16
NL-6700 AA Wageningen
Netherlands
www.primediagnostics.nl

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 5 x 5 cm module (potting soil)

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

Method of inoculation: plants are dusted with carborundum powder on two or 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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Wiebe, H.-J., 1987: Einfluß der Tageslänge auf Entwicklung, Wachstum und Nitratgehalt von Spinatsorten. Gartenbauwissenschaft, 53(3), 103-108.

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	*Spinacea oleracea* L.
1.2 Common name	Spinach

2. Applicant

Name	
Address	
Telephone No.	
Fax No.	
E-mail address	

Breeder (if different from applicant)

3. Proposed denomination and breeder's reference

Proposed denomination (if available)	
Breeder's reference	

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

[#]4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

4.1.1 Crossing

a) controlled cross [ ]
(please state parent varieties)

(b) partially known cross [ ]
(please state known parent variety(ies))

(c) unknown cross [ ]

4.1.2 Mutation [ ]
(please state parent variety)

4.1.3 Discovery and development [ ]
(please state where and when discovered and how developed)

4.1.4 Other [ ]
(please provide details)

4.2 Breeding scheme

(a) Hybrid [ ]
(please state parent varieties)

(b) open-pollinated variety [ ]
(please state known parent variety(ies))

(c) Other [ ]
(please provide details)

[#] 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:

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).

	Characteristics	Example Varieties	Note
5.1 (2)	**Leaf blade: intensity of green color**		
	very light		1[ ]
	light	Monet, Viroflay, Winterreuzen	3[ ]
	medium	Butterflay, Monnopa	5[ ]
	dark	Imola, Lavewa, Nores	7[ ]
	very dark	Lorelay, Mystic	9[ ]
5.2 (3)	**Leaf blade: blistering**		
	absent or very weak	Matador	1[ ]
	weak	Polka, Tarpy	3[ ]
	medium	Butterflay, Koala, Mystic	5[ ]
	strong	Giraffe, Rhythm	7[ ]
	very strong	Menorca, Revolver	9[ ]
5.3 (10)	**Leaf blade: shape of apex**		
	acute	Grappa, Rhythm	1[ ]
	obtuse	Resistoflay	2[ ]
	rounded	Imola, Nores	3[ ]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

	Characteristics	Example Varieties	Note
5.4 (12)	**Proportion of monoecious plants**		
	absent or very low	Medania	1[ ]
	low	Matador	3[ ]
	medium	Figo	5[ ]
	high	Giraffe, Lazio	7[ ]
	very high	Monnopa	9[ ]
5.5 (13)	**Proportion of female plants**		
	absent or very low	Monnopa	1[ ]
	low	Giraffe	3[ ]
	medium	Figo, Medania	5[ ]
	high	Parrot	7[ ]
	very high		9[ ]
5.6 (14)	**Proportion of male plants**		
	absent or very low	Monnopa, Parrot	1[ ]
	low		3[ ]
	medium	Medania	5[ ]
	high		7[ ]
	very high		9[ ]
5.7 (15)	**Time of start of bolting (for spring sown crops, 15% of plants)**		
	very early	Figo, Maracas	1[ ]
	early	Bandola, Viroflay	3[ ]
	medium	Matador, Monnopa	5[ ]
	late	Grappa, Medania, Revolver	7[ ]
	very late	Chica, Lavewa	9[ ]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

6. Similar varieties and differences from these varieties

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.

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
Example	*proportion of male plants*	*absent or very low*	*medium*

Comments:

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

[#]7. Additional information which may help in the examination of the variety

7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

Yes [ ] No [ ]

(If yes, please provide details)

7.2 Are there any special conditions for growing the variety or conducting the examination?

Yes [ ] No [ ]

(If yes, please provide details)

7.3 Other information

(a) Variety use:

– only in glasshouse [ ]
– only in the open [ ]
– in the open and in glasshouse [ ]

(b) Resistance to pests and diseases (specify)

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

Race Pfs: 1	[ ] absent	[ ] present
Race Pfs: 2	[ ] absent	[ ] present
Race Pfs: 3	[ ] absent	[ ] present
Race Pfs: 4	[ ] absent	[ ] present
Race Pfs: 5	[ ] absent	[ ] present
Race Pfs: 6	[ ] absent	[ ] present
Race Pfs: 7	[ ] absent	[ ] present
Race Pfs: 8	[ ] absent	[ ] present
Race Pfs: 10	[ ] absent	[ ] present
Race Pfs: 11	[ ] absent	[ ] present

(ii) Resistance to Cucumber mosaic virus (CMV)

[ ] absent [ ] present

[#] 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:

8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [ ] No [ ]

(b) Has such authorization been obtained?

Yes [ ] No [ ]

If the answer to (b) is yes, please attach a copy of the authorization.

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”.

……

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

Applicant’s name

Signature Date

[End of document]