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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

UNION INTERNATIONALE POUR LA PROTECTION DES OBTENTIONS VÉGÉTALES

INTERNATIONALER VERBAND ZUM SCHUTZ VON PFLANZEN-ZÜCHTUNGEN

UNIÓN INTERNACIONAL PARA LA PROTECCIÓN DE LAS OBTENCIONES VEGETALES

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

SPINACH

(*Spinacea oleracea* L.)

GENEVA
1996

These Guidelines should be read in conjunction with document TG/1/2, which contains explanatory notes on the general principles on which the Guidelines have been established.

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I. Subject of these Guidelines

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

II. Material Required

1. The competent authorities decide when, where and in what quantity and quality the seed required for testing the variety 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 are complied with. The minimum quantity of seed to be supplied by the applicant in one or several samples should be:

250 g.

The seed should at least meet the minimum requirements for germination capacity, moisture content and purity for marketing seed in the country in which the application is made. The germination capacity should be as high as possible.

2. The plant material must not have undergone any treatment unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

III. Conduct of Tests

1. The minimum duration of tests should normally be two similar growing periods.

2. The tests should normally be conducted at one place. If any important characteristics of the variety cannot be seen at that place, the variety may be tested at an additional place.

3. The tests should be carried out under conditions ensuring normal growth. The size of the plots should be such that plants or parts of plants may be removed for measurement and counting without prejudice to the observations which must be made up to the end of the growing period. Each test in the open should include 200 drilled plants and/or 100 single spaced plants. In all cases the total number of plants should be divided between two or more replicates. Separate plots for observation and for measuring can only be used if they have been subject to similar environmental conditions.

4. Additional tests for special purposes may be established.

IV. Methods and Observations

1. All observations determined by measurement or counting should be made on 60 plants or parts of 60 plants.

2. For the assessment of uniformity of hybrid varieties a population standard of 2% with an acceptance probability of at least 95% should be applied to off-types excluding clearly recognizable inbred plants. In the case of a sample size of 200 drilled plants the maximum number of off-types allowed would be 7. In the case of a sample size of 100 spaced plants the maximum number of off-types allowed would be 5. In addition a population standard of 3% with the same acceptance probability should be applied to clearly recognizable inbred plants. In the case of a sample size of 200 drilled plants the additional maximum number of clearly recognizable inbred plants allowed would be 10. In the case of a sample size of 100 spaced plants the additional maximum number of clearly recognizable inbred plants would be 6.

3. Unless otherwise indicated, all 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.

4. All 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.

5. When resistance characteristics are used for assessing distinctness, homogeneity and stability, records must be taken under conditions of controlled infection with the pathotype or isolate specified in the Explanations on the table of characteristics.

V. Grouping of Varieties

1. The collection of varieties to be grown should be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety. Their various states of expression should be fairly evenly distributed throughout the collection.

2. It is recommended that the competent authorities use the following characteristics for grouping varieties:

(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).

VI. Characteristics and Symbols

1. To assess distinctness, homogeneity and stability, the characteristics and their states as given in the Table of Characteristics should be used.

2. Notes (numbers), for the purposes of electronic data processing, are given opposite the states of the different characteristics.

3. Legend:

(*) Characteristics that should be used on all varieties in every growing period over which the examinations are made and always be included in the variety descriptions, except when the state of expression of a preceding characteristic or regional environmental conditions render this impossible.

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

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

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1. (*)	**Seed: spines**	**Semence: épines**	**Samen: Stacheln**	**Semilla: espinas**		
	absent	absentes	fehlend	ausentes	Butterflay	1
	present	présentes	vorhanden	presentes	Bergola, Subito	9
2.	**Seedling: length of cotyledon**	**Plantule: longueur du cotylédon**	**Sämling: Länge des Keimblatts**	**Plántula: longitud del cotiledón**		
	short	court	kurz	corto	Beta, Nores	3
	medium	moyen	mittel	medio		5
	long	long	lang	largo	Breedblad Scherpzaad, Resistoflay, Subito	7
3. (*)	**Leaf blade: intensity of green color**	**Limbe: intensité de la couleur verte**	**Blattspreite: Intensität der Grünfärbung**	**Limbo: intensidad del color verde**		
	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. (*)	**Leaf blade: blistering**	**Limbe: cloqûre**	**Blattspreite: Blasigkeit**	**Limbo: abullonado**		
	absent or very weak	absente ou très faible	fehlend oder sehr gering	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 (*)	**Leaf blade: lobing**	**Limbe: découpure du bord**	**Blattspreite: Lappung**	**Limbo: lobulado**		
	absent or very weak	absente ou très faible	fehlend oder sehr gering	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. (*) (+)	**Petiole: attitude**	**Pétiole: port**	**Blattstiel: Haltung**	**Peciolo: porte**		
	erect	dressé	aufrecht	erecto	Bloomsdale Longstanding	1
	semi-erect	demi-dressé	halbaufrecht	semi-erecto	Monnopa, Prince, Subito	3
	horizontal	horizontal	waagerecht	horizontal	Comte, Lavewa	5
7.	**Petiole: length**	**Pétiole: longueur**	**Blattstiel: Länge**	**Peciolo: longitud**		
	short	court	kurz	corto		3
	medium	moyen	mittel	medio	Butterflay	5
	long	long	lang	largo	Resistoflay	7
8. (*) (+)	**Leaf blade: attitude**	**Limbe: port**	**Blattspreite: Haltung**	**Limbo: porte**		
	erect	dressé	aufrecht	erecto		1
	semi-erect	demi-dressé	halbaufrecht	semi-erecto	Monnopa, Prince, Subito	3
	horizontal	horizontal	waagerecht	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. (*)	**Leaf blade: shape (excluding basal lobes)**	**Limbe: forme (à l'exclusion des lobes basals)**	**Blattspreite: Form (Basallappen ausgenommen)**	**Limbo: forma (excluyendo lóbulos basales)**		
	elliptic	elliptique	elliptisch	elíptica		1
	broad elliptic	elliptique large	breit elliptisch	elíptica ancha	Ass, Comte, Nores	2
	circular	circulaire	rund	circular		3
	ovate	ovale	eiförmig	oval	Lavewa, Prince, Resistoflay	4
	broad ovate	ovale large	breit eiförmig	oval ancha	Butterflay	5
	triangular	triangulaire	dreieckig	triangular	Maracas	6
10.	**Leaf blade: curving of margin**	**Limbe: courbure du bord**	**Blattspreite: Biegung des Randes**	**Limbo: curvado del margen**		
	incurved	incurvé	eingebogen	incurvado	Estivato	1
	flat	plan	flach	plano	Resistoflay	2
	recurved	récurvé	umgebogen	recurvado	Ass	3
11. (*)	**Leaf blade: shape of apex**	**Limbe: forme de la pointe**	**Blattspreite: Form der Spitze**	**Limbo: forma del ápice**		
	acute	aigue	spitz	agudo	Rhythm	1
	obtuse	obtuse	stumpf	obtuso	Prince, Resistoflay, Subito	2
	rounded	arrondie	abgerundet	redondeado	Ass, Comte	3
12. (*)	**Leaf blade: shape in longitudinal section**	**Limbe: forme en section longitudinale**	**Blattspreite: Form im Längsschnitt**	**Limbo: forma en sección longitudinal**		
	concave	concave	konkav	cóncava		1
	flat	plat	flach	plana	Resistoflay	2
	convex	convexe	konvex	convexa	Ass	3

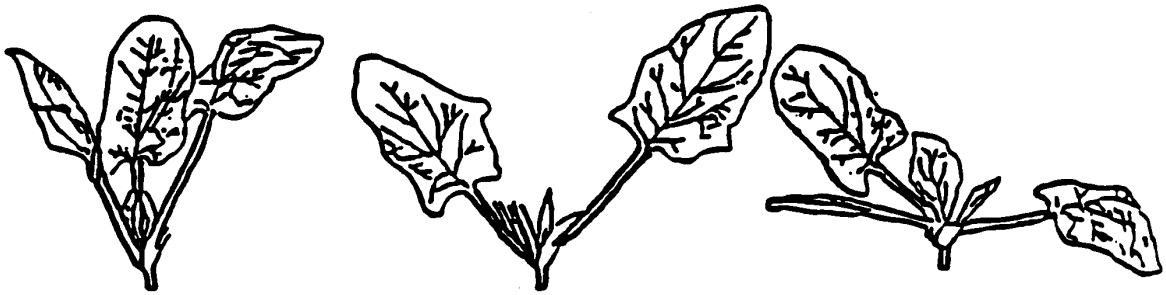
	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
13. (*) (+)	**Flowering plants: proportion of monoecious plants**	**Plantes en floraison: proportion de plantes monoïques**	**Blühende Pflanzen: Anteil monözischer Pflanzen**	**Plantas en floración: proporción de plantas monoicas**		
	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. (*) (+)	**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**		
	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. (*) (+)	**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**		
	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. (*)	**Start of bolting (for spring sown crop, 15% of plants)**	**Début de montaison (pour des variétés semées au printemps, 15% de plantes)**	**Schoßbeginn (bei Frühjahrsaussaat, 15% der Pflanzen)**	**Comienzo del espigado (para cultivos sembrados en primavera, 15% de las plantas)**		
	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. (+)	**Resistance to *Peronospora farinosa* f. *spinaciae***	**Résistance à *Peronospora farinosa* f. *spinaciae***	**Resistenz gegen *Peronospora farinosa* f. *spinaciae***	**Resistencia a *Peronospora farinosa* f. *spinaciae***		
17.1	**Race 1**	**Race 1**	**Pathotyp 1**	**Raza 1**		
	absent	absente	fehlend	ausente	Viroflay, Winterreuzen	1
	present	présente	vorhanden	presente	Condor	9
17.2	**Race 2**	**Race 2**	**Pathotyp 2**	**Raza 2**		
	absent	absente	fehlend	ausente	Master, Medania, Mega	1
	present	présente	vorhanden	presente	Trio, Spokane	9
17.3	**Race 3**	**Race 3**	**Pathotyp 3**	**Raza 3**		
	absent	absente	fehlend	ausente	Subito, Resistoflay	1
	present	présente	vorhanden	presente	Trio, Spokane	9

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
17. **(+)**	**Resistance to *Peronospora farinosa* f. *spinaciae*** **17.4 Race 4**	**Résistance à *Peronospora farinosa* f. *spinaciae*** **Race 4**	**Resistenz gegen *Peronospora farinosa* f. *spinaciae*** **Pathotyp 4**	**Resistencia a *Peronospora farinosa* f. *spinaciae*** **Raza 4**		
	absent	absente	fehlend	ausente	Trio, Spokane	1
	present	présente	vorhanden	presente	Chica, Ballet, Bolero	9
18. **(+)**	**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)**		
	absent	absente	fehlend	ausente	Polka	1
	present	présente	vorhanden	presente	Symphony	9

VIII. Explanation on the Table of Characteristics

Ad. 6: Petiole: attitude

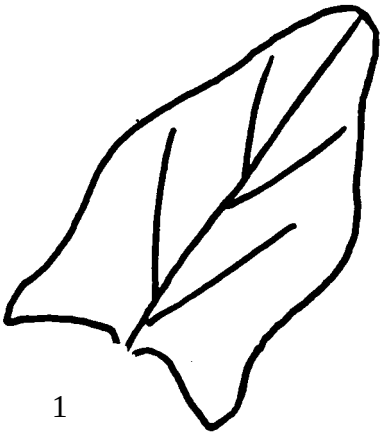


1 erect

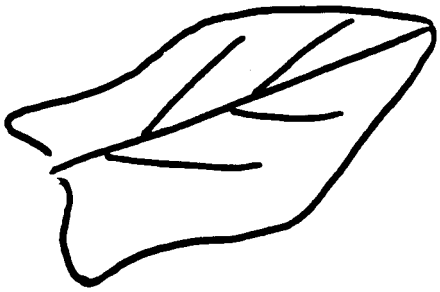
3 semi-erect

5 horizontal

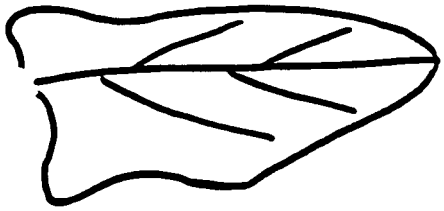
Ad. 8: Leaf blade: attitude



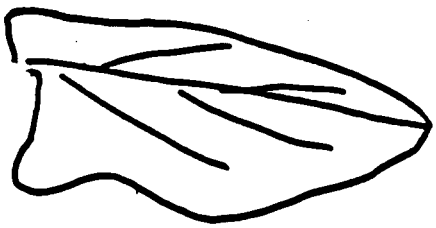
1 erect



3 semi-erect



5 horizontal



7 semi-pendulous

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

	Note	Approximate percentage
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. *spinaciae*

Maintenance of races

Type of medium: Living host plants, obtainable from IPO-DLO, Wageningen, Netherlands

Special conditions: Propagation of separate races on living host plants, inoculation eleven days after sowing, following propagation cycle seven days after first.

In Scheme:

Day 0: sowing for first propagation
Day 7: sowing for second propagation
Day 11: inoculation first propagation
Day 14: sowing of third propagation
Day 18: inoculation second propagation
etc.

Number of host plants and propagations according to needs.
Resistance controls are included in the propagation cycle.

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: Host plants and test plants are grown on modules of pot soil in glasshouse.

Method of inoculation: The infected leaves, taken from host plants that were infected seven days before, are washed in as little water as possible (maximum 150 ml water per 224 plants).
This suspension is filtered through cheese cloth. With 150 ml of suspension a maximum of 3 x 224 plants are infected.
Spore density is 20.000 to 100.000 conidia/ml water.
Suspension must be immediately sprayed over the test plants to ensure the vigor of the conidia. The leaves from the test plant should be wet, but no suspension should be dripping from the soil.

Remarks: 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:

- Sowing to inoculation: 11 days.
- Inoculation to reading: 10 days.

Number of plants tested: 56 plants.

Evaluation of infection: Sporangia can be found on the lower side and later on the upper side of the leaves of susceptible plants.

Control varieties to identify races:

race 1	Susceptible	Viroflay, Winterreuzen
	Resistant	Condor
race 2	Susceptible	Master, Medania, Mega
	Resistant	Trio, Spokane
race 3	Susceptible	Subito, Resistoflay
	Resistant	Trio, Spokane
race 4	Susceptible	Trio, Spokane
	Resistant	Chica, Ballet, Bolero

Ad 18: Resistance to Cucumber mosaic virus (CMV)

Maintenance of isolate

Storage of medium:	on leaves in freezer or desiccated over $CaCl_2$
Special conditions:	Isolates NL 16 and SP 43 obtainable at IPO-DLO, Wageningen, Netherlands
Maintenance and propagation of pathotypes:	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 innoculum:	the 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

IX. Literature

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X. Technical Questionnaire

	Reference Number (not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE
to be completed in connection with an application for plant breeders' rights

1.	Species	*Spinacea oleracea* L. SPINACH
2.	Applicant (Name and address)	
3.	Proposed denomination or breeder's reference	

4. Information on origin, maintenance and reproduction of the variety

4.1 Method of maintenance and reproduction

(a) Hybrid [ ]

(b) open-pollinated variety [ ]

4.2. Other information

5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the state of expression which best corresponds).

	Characteristics	Example Varieties	Note
5.1 (1)	**Seed: spines**		
	absent	Butterflay	1[ ]
	present	Bergola, Subito	9[ ]
5.2 (3)	**Leaf blade: intensity of green color**		
	very light	Virtuosa	1[ ]
	light	Subito	3[ ]
	medium	Butterflay, Monnopa	5[ ]
	dark	Larewa, Trinidad, Wobli	7[ ]
	very dark	Lorelay	9[ ]

	Characteristics	Example Varieties	Note
5.3 (4)	**Leaf blade: blistering**		
	absent or very weak		1[ ]
	weak	Polka, Prince, Vital	3[ ]
	medium	Beta, Butterflay	5[ ]
	strong	Martine, Rhythm	7[ ]
	very strong	Bloomsdale Longstanding	9[ ]
5.4 (11)	**Leaf blade: shape of apex**		
	acute	Rhythm	1[ ]
	obtuse	Prince, Resistoflay, Subito	2[ ]
	rounded	Ass, Comte	3[ ]
5.5 (13)	**Flowering plants: proportion of monoecious plants**		
	absent or very low	Medania, Ass	1[ ]
	low	Comte, Matador	3[ ]
	medium	Spencer	5[ ]
	high	Beta	7[ ]
	very high	Monnopa, Trinidad	9[ ]
5.6 (14)	**Flowering plants: proportion of female plants**		
	absent or very low	Monnopa, Trinidad	1[ ]
	low	Beta, Comte	3[ ]
	medium	Medania, Spencer	5[ ]
	high		7[ ]
	very high		9[ ]

	Characteristics	Example Varieties	Note
5.7 (15)	**Flowering plants: proportion of male plants**		
	absent or very low	Beta, Monnopa, Trinidad	1[ ]
	low		3[ ]
	medium	Ass, Comte, Medania	5[ ]
	high		7[ ]
	very high		9[ ]
5.8 (16)	**Start of bolting (for spring sown crop, 15% of plants)**		
	very early		1[ ]
	early	Subito	3[ ]
	medium	Monnopa	5[ ]
	late	Meania, Wobli	7[ ]
	very late	Chica, Spencer, Lavewa	9[ ]

6. Similar varieties and differences between these varieties

Denomination of similar variety	Characteristic in which the similar variety is different o)	State of expression of similar variety	State of expression of candidate variety

o) In the case of identical states of expressions of both varieties, please indicate the size of the difference.

7. Additional information which may help to distinguish the variety

7.1 Resistance to pests and diseases

		absent	present	not tested
(a)	*Peronospora farinosa* f. *spinacea*			
	Race 1 (characteristic 17.1)	[ ]	[ ]	[ ]
	Race 2 (characteristic 17.2)	[ ]	[ ]	[ ]
	Race 3 (characteristic 17.3)	[ ]	[ ]	[ ]
	Race 4 (characteristic 17.4)	[ ]	[ ]	[ ]
(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 and in glasshouse [ ]

(b) Other conditions

.....

7.3 Other information

[End of document]