Calibration book

Spinacea oleracea L.

Spinach

Naktuinbouw calibration book

Spinacea oleracea L.

spinach

Introduction

In front of you, you find the calibration book for spinach. This book may be used as guidance for the completion of application forms, the describing of varieties or the understanding of variety descriptions. This book can not replace the skill needed to make a variety description, but may serve as support.

Sources used
The basis for this book is the CPVO protocol TP/055/3 which in turn is based on UPOV Guideline TG/55/7. Please also use these sources for reference when using this calibration book. The application of this calibration book is based on the general UPOV principles on the definitions and use of characteristics of variety descriptions (UPOV TG/1/3).

Application methodology
The UPOV system is based on the expression of characteristics that are related to the expression values of example varieties. In the calibration book you find two types of characteristics; visually assessed characteristics and measured characteristics.
The value of the visually assessed characteristics can be compared with the visual value of the expression of example varieties. In the calibration book you may find drawings or pictures to assist in the decision on the applicable expression. For measured characteristics this is more complicated as in many cases the value of the measurements is depending on the (climatical) conditions of the trials. The use of example varieties in these cases is indispensable. The same applies for those visually assessed characteristics that are prone to influence by climate (e.g. anthocyanin coloration). In this calibration book these example varieties are only included for the characteristics that appear in the Technical Questionnaire. Others are not included as many prefer their own set of example varieties, but may be found in the relevant CPVO protocol.

Website
The CPVO and UPOV documents mentioned above can be found on the Naktuinbouw website (*http://www.naktuinbouw.nl/onderwerp/kalibratieboeken*). On this website you can also find announcements of possible modifications of the published calibration books.

Helpdesk
For possible remarks, suggestions and questions on the calibration books and the website, you may contact Naktuinbouw at our email address: kalibratieboek@naktuinbouw.nl

Contents

1 Seed: spines (submitted seed)

Grouping characteristic: no.

Type of characteristic: QL – Qualitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: On submitted seeds.

Method of observation: Visual observation. Seeds without spines are rounded. Seeds with spines are pointed and sharp. Calibrate using the explanatory photos.

Notes and states of expression:
1: absent
9: present
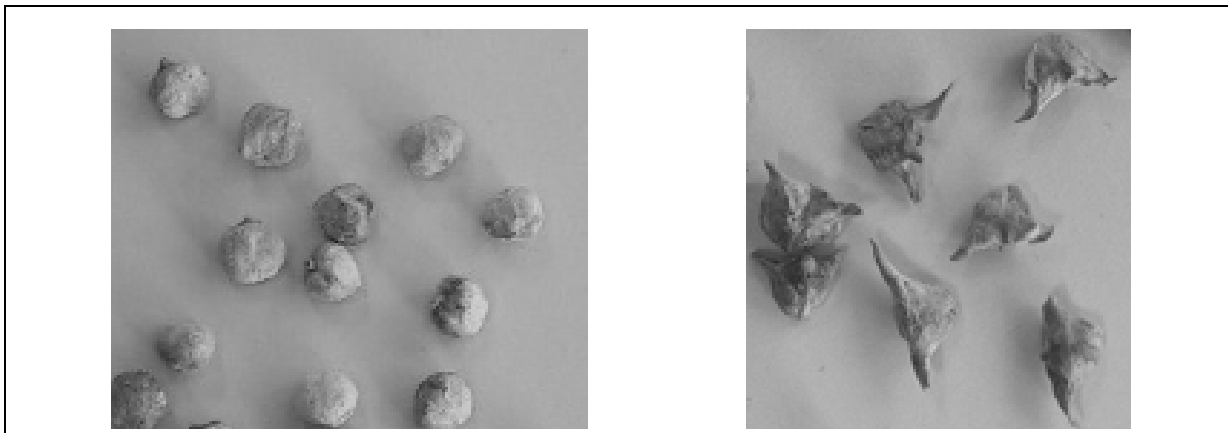
| 1: absent | 9: present |

2 Seedling: length of cotyledon

Grouping characteristic: no.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: At full development of the cotyledons, just when the development of the first leaves start. This is approximately a week after sowing. It might be necessary to observe the varieties at different times because the pace of emergence and growth of seedlings can vary.

Method of observation: Visual observation. Calibrate using example varieties.

Notes and states of expression:
1: very short
2: very short to short
3: short
4: short to medium
5: medium
6: medium to long
7: long
8: long to very long
9: very long

3 Leaf blade: intensity of green colour

Grouping characteristic: yes.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: On the seventh to tenth leaf of the adult not bolted plant.

Method of observation: Visual observation. Calibrate using example varieties.

Notes, states of expression and example varieties:
1: very light
2: very light to light
3: light   Monet, Viroflay, Winterreuzen
4: light to medium
5: medium   Butterflay, Monnopa
6: medium to dark
7: dark   Imola, Lavewa, Nores
8: dark to very dark
9: very dark   Lorelay, Mystic

3 Leaf blade: intensity of green colour

Diversity in intensity of green colour.

These images serve only to illustrate the variation present in the crop and should not be used as an absolute reference.

4 Leaf blade: blistering

Grouping characteristic: yes.

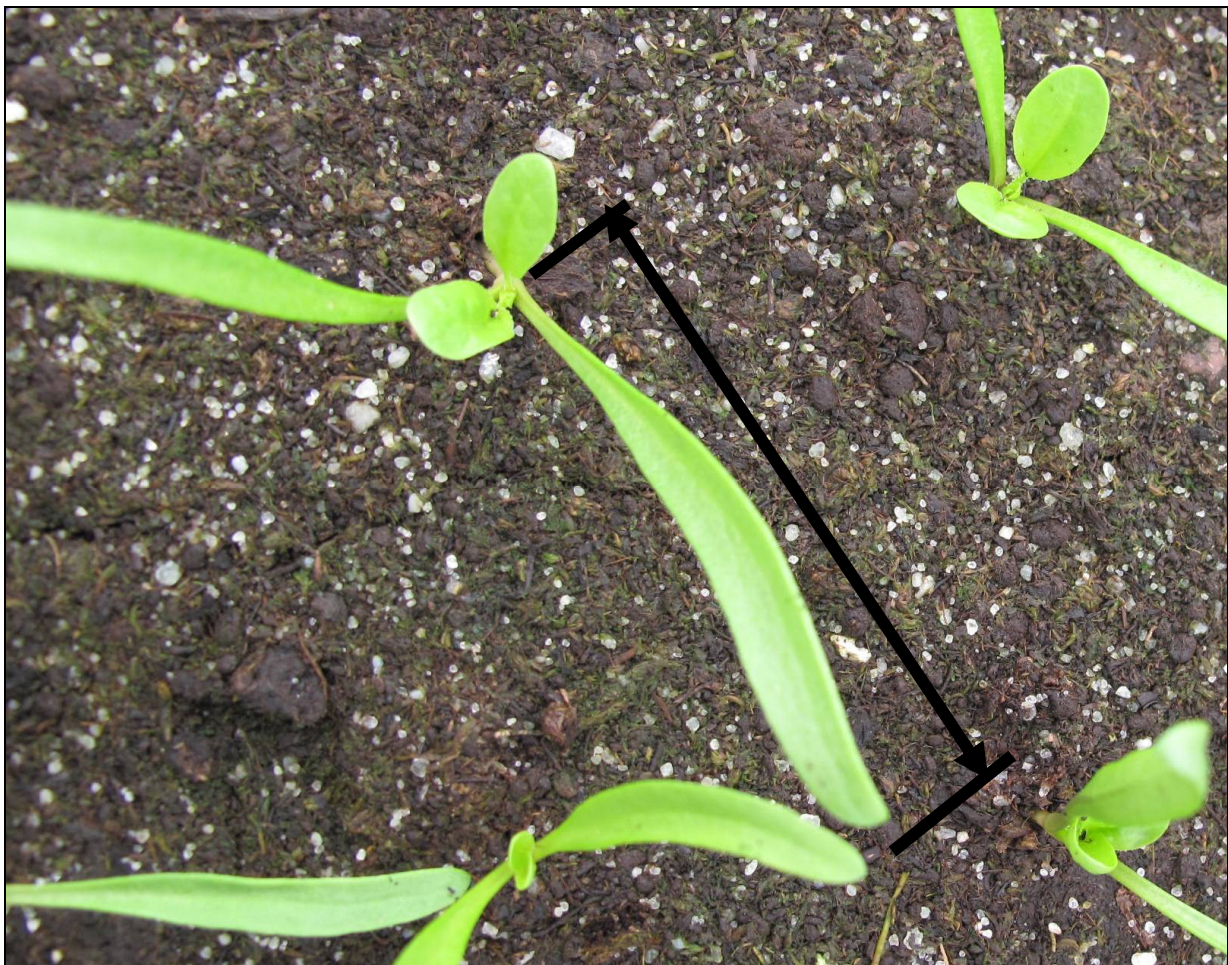**Type of characteristic: QN** – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: On the seventh to tenth leaf of the adult not bolted plant.

Method of observation: Visual observation. Note: this involves the number of blisters and not the size of the blisters. The leaf margin is often less blistered than the other parts of the leaf, but to the center of the leaf it is sometimes difficult to distinguish the blisters from creasing (i.e. the longitudinal undulation of the leaf surface parallel to the veins). Do not describe creasing. Some varieties start to bolt at an early stage. Be aware, especially with regard to those varieties, to observe only the leaves attached to the main stem. Calibrate using example varieties and explanatory photos.

Notes, states of expression and example varieties:
1: absent or very weak   Matador
2: very weak to weak
3: weak   Polka, Tarpy
4: weak to medium
5: medium   Butterflay, Koala, Mystic
6: medium to strong
7: strong   Giraffe, Rhythm
8: strong to very strong
9: very strong   Menorca, Revolver

4 Leaf blade: blistering

Diversity in blistering.

These images serve only to illustrate the variation present in the crop and should not be used as an absolute reference.

5 Leaf blade: lobing

Grouping characteristic: no.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: On the seventh to tenth leaf of the adult not bolted plant.
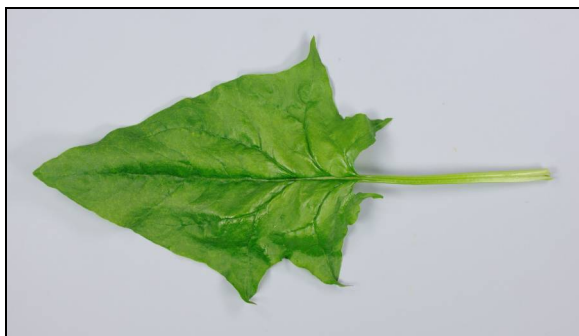
Method of observation: Visual observation. The incisions between secondary veins form the lobes at the side of the petiole. The lobing is stronger when the incisions are deeper (and sometime more numerous). Some varieties start to bolt at an early stage. Be aware, especially with regard to those varieties, to observe only the leaves attached to the main stem. Calibrate using example varieties and explanatory drawings and photos.

 Notes and states of expression:
1: absent or very weak
2: absent or very weak to weak
3: weak
4: weak to medium
5: medium
6: medium to strong
7: strong
8: strong to very strong
9: very strong

CPVO explanation:

5 Leaf blade: lobing

3: weak
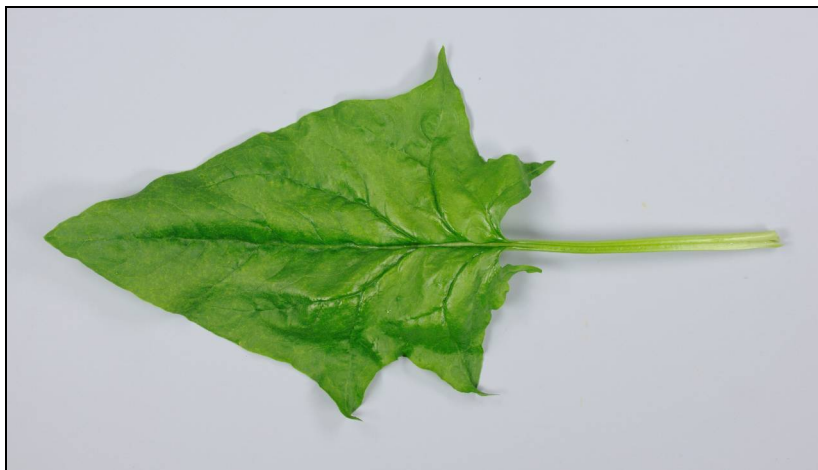
5: medium

9: very strong

5 Leaf blade: lobing

6 Petiole: attitude

Grouping characteristic: no.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: On the seventh to tenth leaf of the adult not bolted plant.

Method of observation: Visual observation. Some varieties start to bolt at an early stage. Be aware, especially with regard to those varieties, to observe only the leaves attached to the main stem. Calibrate using example varieties and explanatory drawings.

Notes and states of expression:
1: erect
2: erect to semi-erect
3: semi-erect
4: semi-erect to horizontal
5: horizontal

CPVO explanation:

7 Petiole: length

Grouping characteristic: no.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.
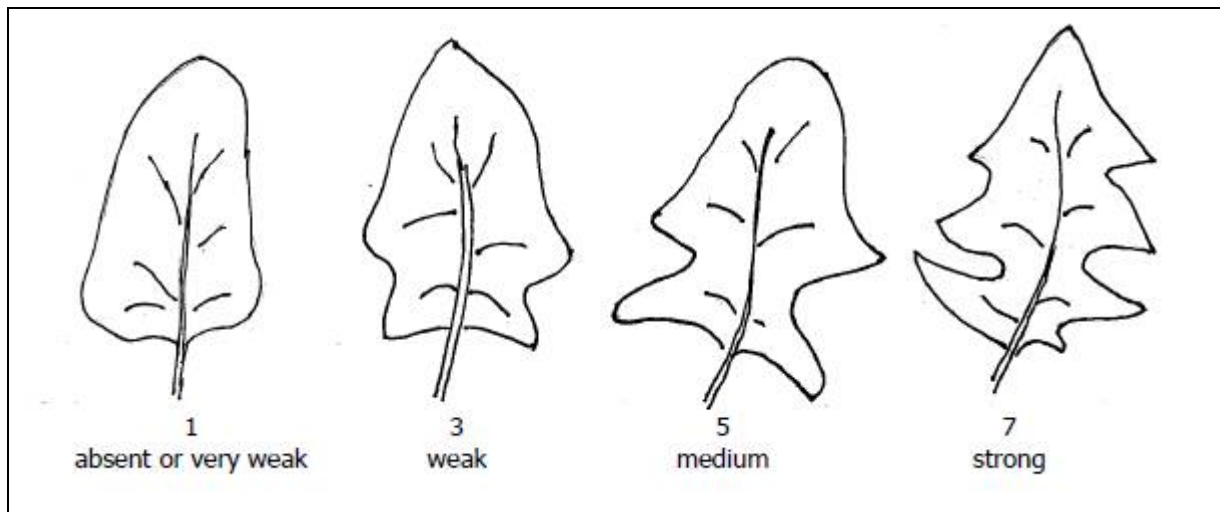
Stage of observation: On the seventh to tenth leaf of the adult not bolted plant.

Method of observation: Visual observation. Some varieties start to bolt at an early stage. Be aware, especially with regard to those varieties, to observe only the leaves attached to the main stem. Calibrate using example varieties and explanatory photos.

Notes and states of expression:
1: very short
2: very short to short
3: short
4: short to medium
5: medium
6: medium to long
7: long
8: long to very strong
9: very long

7: long   5: medium   3: short

8 Leaf blade: attitude

Grouping characteristic: no.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.
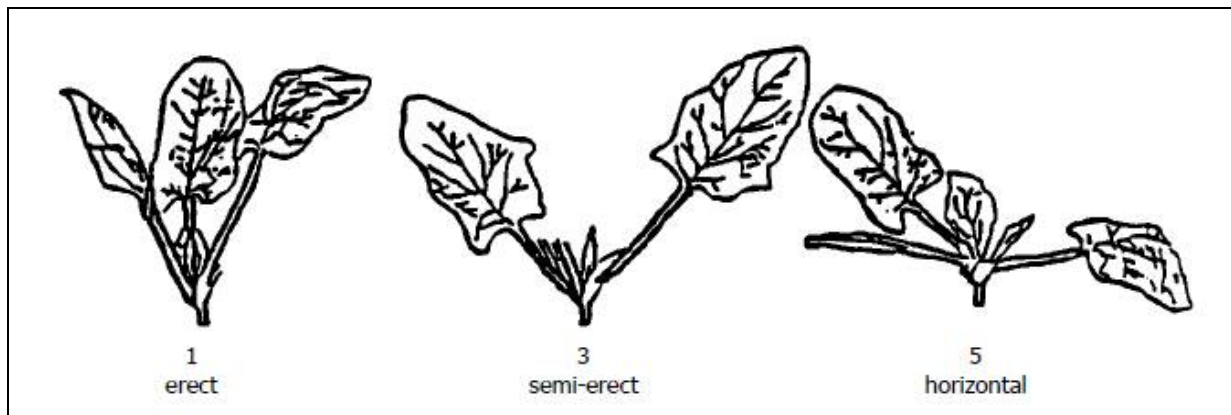
Stage of observation: On the seventh to tenth leaf of the adult not bolted plant. Some varieties start to bolt at an early stage. Be aware, especially with regard to those varieties, to observe only the leaves attached to the main stem.

Method of observation: Visual observation. Calibrate using example varieties and explanatory drawings.

Notes and states of expression:
1: erect
2: erect to semi-erect
3: semi-erect
4: semi-erect to horizontal
5: horizontal
6: horizontal to semi-pendulous
7: semi-pendulous

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

9 Leaf blade: shape (excluding basal lobes)

Grouping characteristic: no.

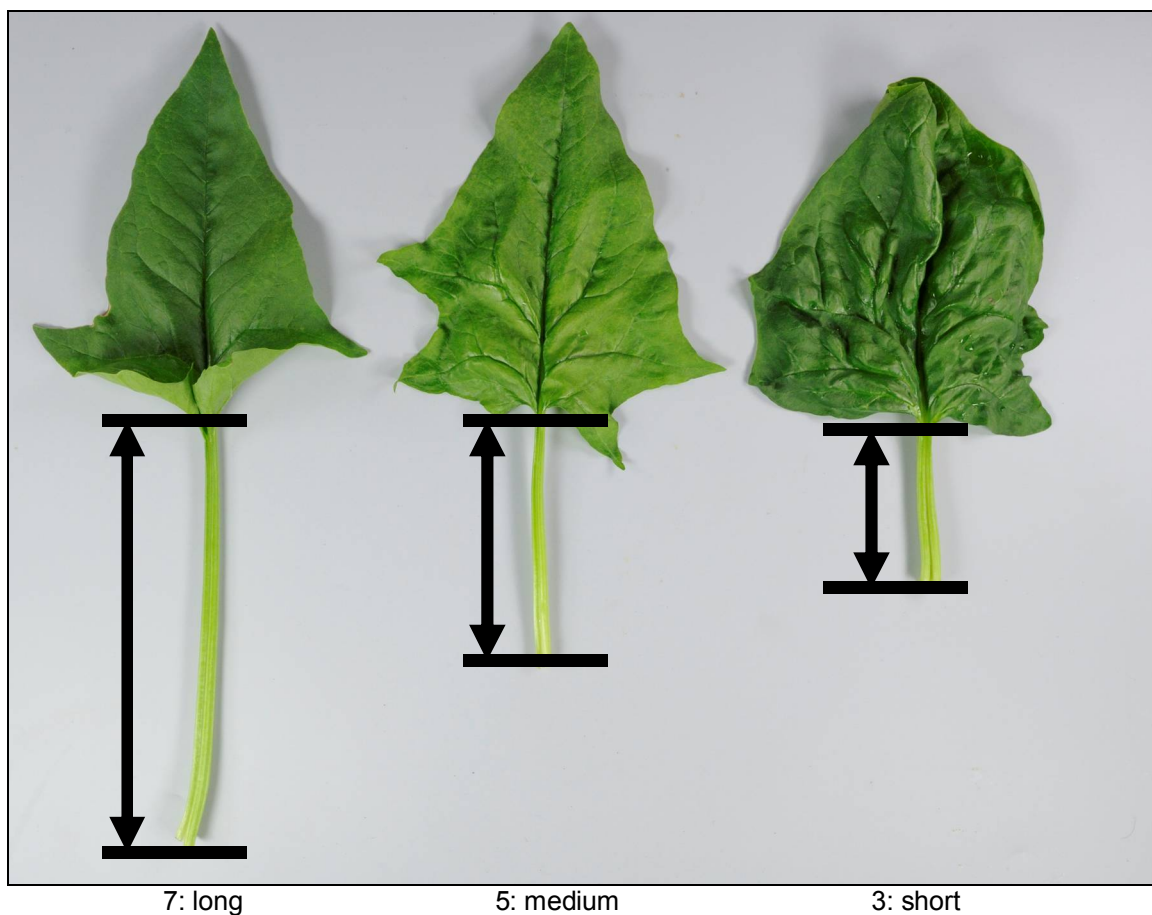**Type of characteristic: PQ** – Pseudo-qualitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: Observations on the seventh to tenth leaf of the adult not bolted plant. Some varieties start to bolt at an early stage. Be aware, especially with regard to those varieties, to observe only the leaves attached to the main stem.

Method of observation: Visual observation. Calibrate using example varieties and explanatory photos.

Notes and states of expression:
1: triangular
2: medium ovate
3: broad ovate
4: medium elliptic
5: broad elliptic
6: circular

9 Leaf blade: shape (excluding basal lobes)

1: triangular

2: medium ovate

3: broad ovate

4: medium elliptic

5: broad elliptic

6: circular

10 Leaf blade: curving of margin

Grouping characteristic: no.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: Observations on the leaf blade should be made on the seventh to tenth leaf of the adult not bolted plant. Some varieties start to bolt at an early stage. Be aware, especially with regard to those varieties, to observe only the leaves attached to the main stem.
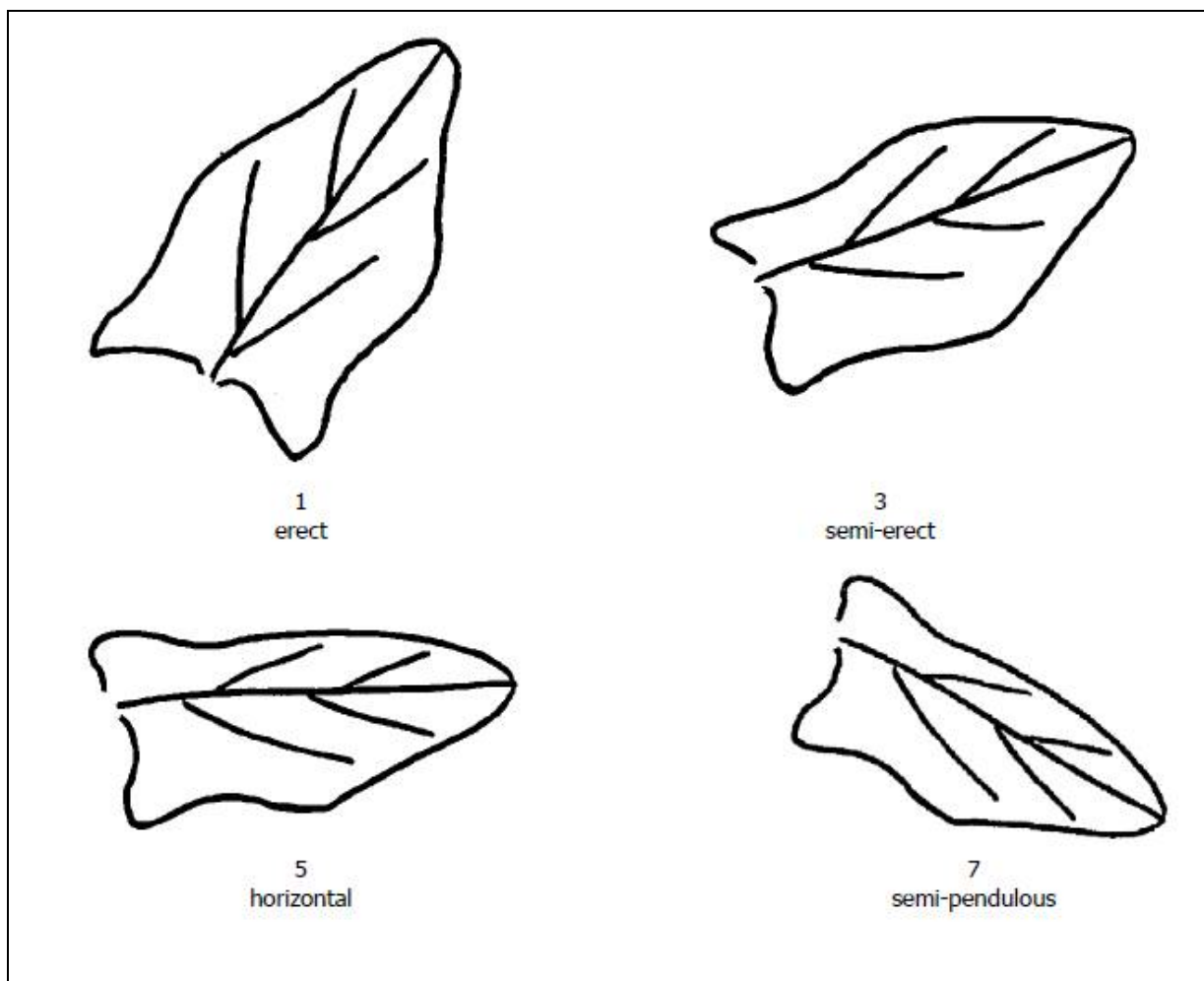
Method of observation: Visual observation. Calibrate using example varieties and explanatory photos.

Notes and states of expression:
1: incurved
2: flat
3: recurved

10 Leaf blade: curving of margin
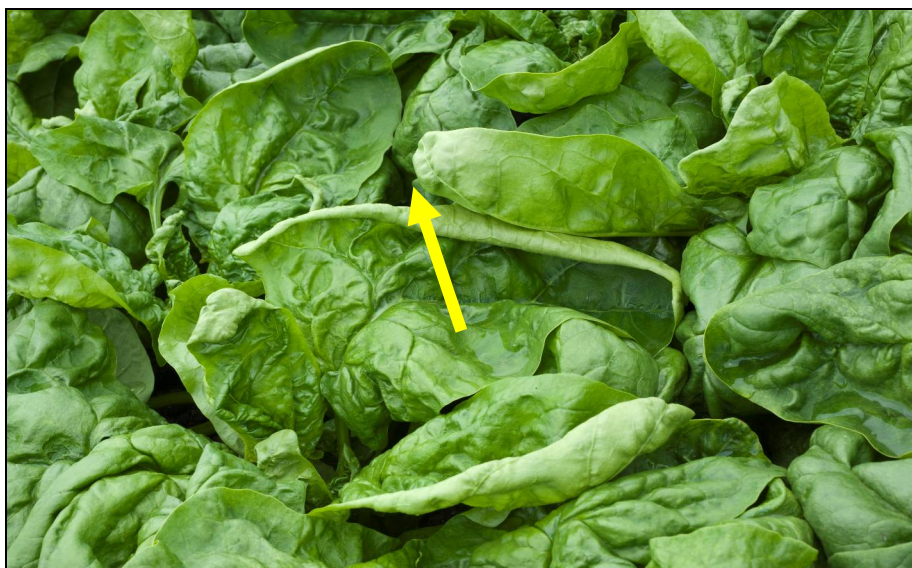
1: incurved

2: flat

3: recurved

11 Leaf blade: shape of apex

Grouping characteristic: no.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: On the seventh to tenth leaf of the adult not bolted plant. Some varieties start to bolt at an early stage. Be aware, especially with regard to those varieties, to observe only the leaves attached to the main stem.

Method of observation: Visual observation. Calibrate using example varieties and explanatory photos.

Notes, states of expression and example varietties:
1: acute   Grappa, Rhythm
2: obtuse   Resistoflay
3: rounded   Imola, Nores

11 Leaf blade: shape of apex

1: acute   Grappa, Rhythm

2: obtuse   Resistoflay

3: rounded   Imola, Nores

11 Leaf blade: shape of apex

12 Leaf blade: shape in longitudinal section

Grouping characteristic: no.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: VG – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: On the seventh to tenth leaf of the adult not bolted plant. Some varieties start to bolt at an early stage. Be aware, especially with regard to those varieties, to observe only the leaves attached to the main stem. Also take note that this characteristic is about the whole surface of the leaf from apex to petiole. The curving of the margin (characteristic 10) is to be excluded.

Method of observation: Visual observation. Calibrate using example varieties and explanatory photos.

Notes and states of expression:
1: concave
2: flat
3: convex

12. Leaf blade: shape in longitudinal section

1: concave
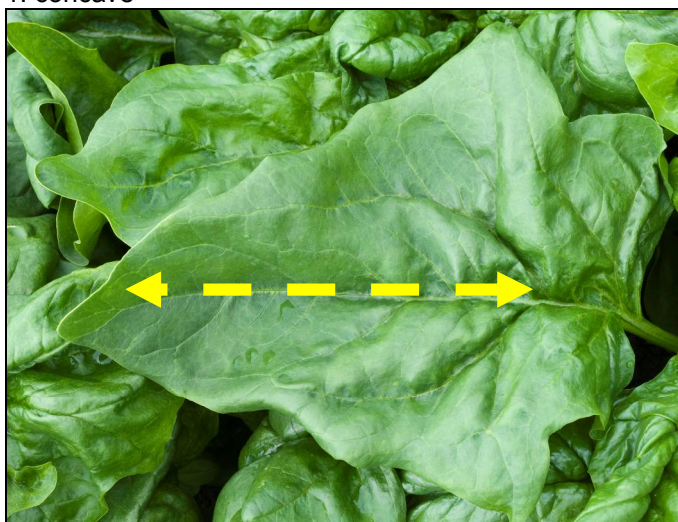
2: flat
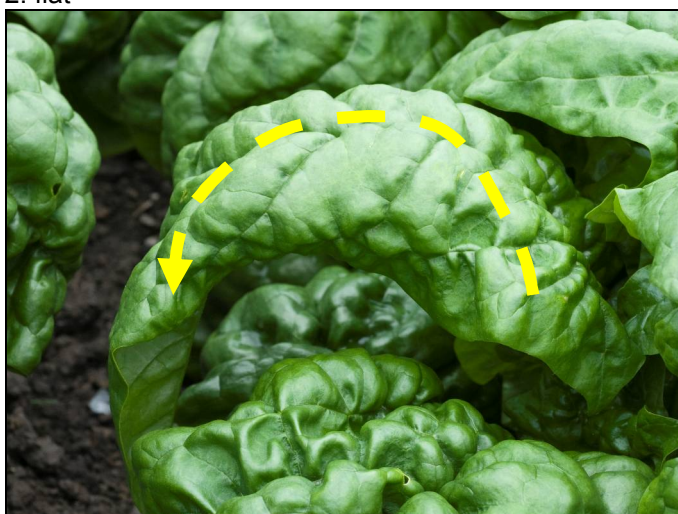
3: convex

13 Proportion of monoecious plants

Grouping characteristic: yes.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: **VS** – Calculated number based on individual assessments of 60 plants or parts of plants.

Stage of observation: Observations on the proportion of monoecious plants should be made at the beginning of seed setting.

Method of observation:
Estimate the percentage of plants which have both male flowers and female flowers on each plant. The ovaries and stigmas of the female flowers must be clearly visible. The perianth of the female flower later grows out to a hard shell: the seed. The male flowers have green anthers which swell and turn yellow a day before anthesis. This observation has to be done on a minimum of 60 plants.

Note: Proportion of monoecious plants, female plants (characteristic 14) and male plants (characteristic 15), out of one sample of as a minimum of 60 plants, has to comprise a total of 100%. It is therefore easier to combine observations of those three characteristics.

Notes, states of expression and example varieties:

1: absent or very low	(<10%)	Medania
2: very low to low	(20%)	
3: low	(30%)	Matador
4: low to medium	(40%)	
5: medium	(50%)	Figo
6: medium to high	(60%)	
7: high	(70%)	Giraffe, Lazio
8: high to very high	(80%)	
9: very high	(>90%)	Monnopa

monoecious plants

ovary

filament
anther   **stamen**

14 Proportion of female plants

Grouping characteristic: yes.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: **VS** – Calculated number based on individual assessments of 60 plants or parts of plants.

Stage of observation: Observations on the proportion of female plants should be made at the beginning of seed setting.

Method of observation:
Estimate a percentage of plants which have only female flowers. The ovaries and stigmas of the female flowers must be clearly visible The perianth of the female flower later grows out to a hard shell: the seed. This observation has to be done on a minimum of 60 plants.

Note: Proportion of female plants, monoecious plants (characteristic 13) and male plants (characteristic 15) has to be together 100%. It's therefore easier to combine observations of those three characteristics.

Notes, states of expression and example varieties:
1: absent or very low   (<10%)   Monnopa
2: very low to low   (20%)
3: low   (30%)   Giraffe
4: low to medium   (40%)
5: medium   (50%)   Figo, Medania
6: medium to high   (60%)
7: high   (70%)   Parrot
8: high to very high   (80%)
9: very high   (>90%)

Female plants.   **ovaries**

15 Proportion of male plants

Grouping characteristic: yes.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: **VS** – Calculated number based on individual assessments of 60 plants or parts of plants.

Stage of observation: Observations on the proportion of male plants should be made at the beginning of seed setting.

Method of observation:
Estimate a percentage of plants which have only male flowers. The male flowers have green anthers which swell and turn yellow a day before anthesis. This observation has to be done on a minimum of 60 plants.

Note: Proportion of male plants, monoecious plants (characteristic 13) and female plants (characteristic 14) has to be together 100%. It's therefore easier to combine observations of those three characteristics.

Notes, states of expression and example varieties:
1: absent or very low   (<10%)   Monnopa, Parrot
2: very low to low   (20%)
3: low   (30%)
4: low to medium   (40%)
5: medium   (50%)   Medania
6: medium to high   (60%)
7: high   (70%)
8: high to very high   (80%)
9: very high   (>90%)

Male plants.

16 Time of start of bolting (for spring sown crops, 15% of plants)

Grouping characteristic: yes.

Type of characteristic: QN – Quantitative characteristic.

Type of observation: **MG** – Single measurement of a group of plants or parts of plants; in practice a single measurement of an average single plant or part of plant.

Stage of observation: Observation after the vegetative phase. From the day the first plant of a sample is starting to bolt until all the plants of the sample are starting to bolt. The time of bolting of a plant is when the central flowering stem appears through stretching of the internodes

Method of observation: Observation on all individual plants of a sample, preferably three times a week. Record the date when a plant is starting to bolt. Calibrate using example varieties.

Notes, states of expression and example varieties:
1: very early   Figo, Maracas
2: very early to early
3: early   Bandola, Viroflay
4: early to medium
5: medium   Matador, Monnopa
6: medium to late
7: late   Grappa, Medania, Revolver
8: late to very late
9: very late   Chica, Lavewa

Central flowering stem.

17 Seed: spines (harvested seed)

Grouping characteristic: no.

Type of characteristic: QL – Qualitative characteristic.

Type of observation: **VG** – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Stage of observation: Observation made on the harvested seeds.

Method of observation: Observation on the seeds to be harvested on the plants grown of the submitted seeds, to see if the spines are absent or present. Seeds without spines are rounded. Seeds with spines are pointed and sharp.

Note: in case of seeds with spines, the spines might be sharp; it can be useful to use some gloves to study the plants.

 Notes and states of expression:
1: absent
2: present

1: absent

2: present

18 Resistance to *Peronospora farinose* **f. sp. s***pinaciae*

Type of characteristic: QL – Qualitative characteristic.

Type of observation: **VG** – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.
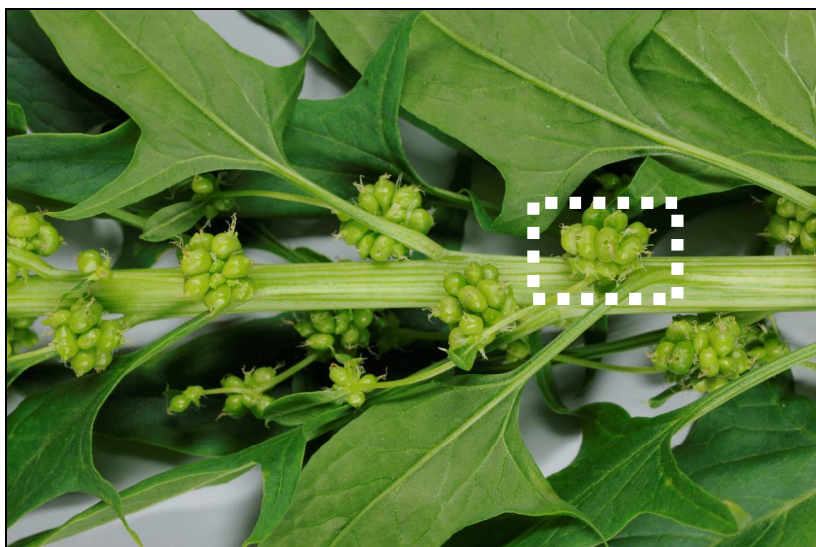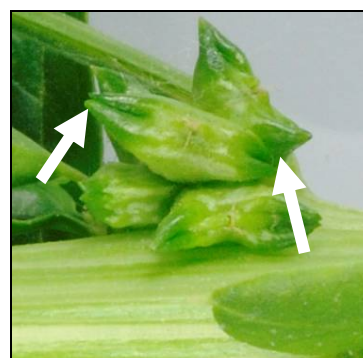
Method of observation: see test protocol

18.1 Resistance to *Peronospora farinose* **f. sp. s***pinaciae* **Race Pfs: 1**

Grouping characteristic: no.

 Notes and states of expression:
1: absent
2: present

18.2 Resistance to *Peronospora farinose* **f. sp. s***pinaciae* **Race Pfs: 2**

Grouping characteristic: no.

 Notes and states of expression:
1: absent
2: present

18.3 Resistance to *Peronospora farinose* **f. sp. s***pinaciae* **Race Pfs: 3**

Grouping characteristic: no.

 Notes and states of expression:
1: absent
2: present

18.4 Resistance to *Peronospora farinose* **f. sp. s***pinaciae* **Race Pfs: 4**

Grouping characteristic: no.

 Notes and states of expression:
1: absent
2: present

18.5 Resistance to *Peronospora farinose* **f. sp. s***pinaciae* **Race Pfs: 5**

Grouping characteristic: yes.

 Notes, states of expression and example varieties:
1: absent   Clermont
2: present   Califlay, Campania

18.6 Resistance to *Peronospora farinose* **f. sp. s***pinaciae* **Race Pfs: 6**

Grouping characteristic: yes.

 Notes, states of expression and example varieties:
1: absent   Califlay, Campania
2: present   Boeing

18.7 Resistance to *Peronospora farinose* f. sp. s*pinaciae* Race Pfs: 7

Grouping characteristic: yes.

 Notes, states of expression and example varieties:
1: absent   Califlay
2: present   Campania

18.8 Resistance to *Peronospora farinose* f. sp. s*pinaciae* Race Pfs: 8

Grouping characteristic: no.

 Notes and states of expression:
1: absent
2: present

18.9 Resistance to *Peronospora farinose* f. sp. s*pinaciae* Race Pfs: 10

Grouping characteristic: no.

 Notes and states of expression:
1: absent
2: present

18.10 Resistance to *Peronospora farinose* f. sp. s*pinaciae* Race Pfs: 11

Grouping characteristic: no.

 Notes and states of expression:
1: absent
2: present

18 Resistance to *Peronospora farinose* **f. sp.** s*pinaciae*

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 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
Viroflay	S	S	S	S	S	S	S	S	S
Resistoflay	R	R	S	S	S	S	S	S	S
Califlay	R	S	R	S	R	S	S	R	S
Clermont	R	R	R	R	S	S	S	S	S
Campania	R	R	R	R	R	S	R	S	S
Boeing	R	R	R	R	R	R	R	S	S
Lion	R	R	R	R	R	R	R	R	S
Lazio	R	R	R	R	R	R	R	R	R

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

19 Resistance to Cucumber mosaic virus (CMV)

Grouping characteristic: no.

Type of characteristic: QL – Qualitative characteristic.

Type of observation: **VG** – Single visual assessment of a group of plants or parts of plants; in practice a single assessment of an average single plant or part of plant.

Method of observation: see test protocol

 Notes and states of expression:
1: absent
2: present

19 Resistance to Cucumber mosaic virus (CMV)

19 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

Notes