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# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

**SUNFLOWER**

UPOV Code(s): HLNTS_ANN

*Helianthus annuus* L.

**GUIDELINES**

**FOR THE CONDUCT OF TESTS**

**FOR DISTINCTNESS, UNIFORMITY AND STABILITY**

*prepared by experts from Hungary*
*to be considered by the*
*Technical Working Party for Agricultural Crops*
*at its fifty-first session, to be held in Cambridge, United Kingdom,*
*from 2022-05-23 to 2022-05-27*

*Disclaimer: this document does not represent UPOV policies or guidance*

Alternative names:*

| *Botanical name* | *English* | *French* | *German* | *Spanish* |
|---|---|---|---|---|
| *Helianthus annuus* L. | Common Sunflower | Soleil, Tournesol | Sonnenblume | Girasol |

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 *Helianthus annuus* L. (excluding ornamental varieties).

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 ensure 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, to be supplied by the applicant, should be:

5000 grains for inbred lines
1 kg for hybrid and open-pollinated varieties

In the case of hybrid varieties, an additional 5000 grains of each component (e.g. for a single hybrid, the female lines (male sterile line and maintainer line) and the male line) should be submitted. In the case of male sterile lines, an additional 5000 grains of the maintainer line should be submitted.

The seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority. In cases where the seed is to be stored, the germination capacity should be as high as possible and should, be stated by the applicant.

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*

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

3.1.2 The two independent growing cycles should be in the form of two separate plantings.

3.1.3 The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test.

*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 optimum stage of development for the assessment of each characteristic is indicated by a number in the Table of Characteristics. The stages of development denoted by each number are described in Chapter 8.

*3.4 Test Design*

3.4.1 Each test should be designed to result in a total of at least 40 plants, which should be divided between at least 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 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.

To assess distinctness of hybrids, the parent lines and the formula may be used according to the following recommendations:

(i) description of parent lines according to the Test Guidelines;

(ii) check of the originality of the parent lines in comparison with the variety collection, based on the characteristics in Chapter 7, in order to identify similar parent lines;

(iii) check of the originality of the hybrid formula in relation to the hybrids in the variety collection, taking into account the most similar lines; and

(iv) assessment of the distinctness at the hybrid level for varieties with a similar formula.

Further guidance is provided in documents TGP/9 "Examining Distinctness" and TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability".

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.

4.1.4 Number of Plants or Parts of Plants to be Examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 36 plants or parts of plants taken from each of 36 plants and any other observations made on all plants in the test, disregarding any off-type plants.

4.1.5 Method of Observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the Table of Characteristics (see document TGP/9 “Examining Distinctness”, Section 4 “Observation 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

Type of observation: visual (V) or measurement (M)

“Visual” observation (V) is an observation made on the basis of the expert’s judgment. For the purposes of this document, “visual” observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. color charts). Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

Type of record: for a group of plants (G) or for single, individual plants (S)

For the purposes of distinctness, observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded as records for a number of single, individual plants or parts of plants (S). In most cases, “G” provides a single record per variety and it is not possible or necessary to apply statistical methods in a plant-by-plant analysis for the assessment of distinctness.

In cases where more than one method of observing the characteristic is indicated in the Table of Characteristics (e.g. VG/MG), guidance on selecting an appropriate method is provided in document TGP/9, Section 4.2.

*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 These Test Guidelines have been developed for the examination of seed-propagated varieties. For varieties with other types of propagation, the recommendations in the General Introduction and document TGP/13 "Guidance for new types and species" Section 4.5 "Testing Uniformity" should be followed.

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

4.2.4 The assessment of uniformity for hybrid varieties depends on the type of hybrid and should be according to the recommendations for hybrid varieties in the General Introduction.

4.2.5 Where the assessment of a hybrid variety involves the parent lines, the uniformity of the hybrid variety should, in addition to an examination of the hybrid variety itself, also be assessed by examination of the uniformity of its parent lines.

4.2.6 For the assessment of uniformity of inbred lines, a population standard of 2% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 36 plants, 2 off-types are allowed.

In addition, the same population standard and acceptance probability should apply for the assessment of uniformity regarding out-crosses and isogenic male fertile plants in a male sterile line. For the assessment of uniformity of single hybrids, a population standard of 5% with an acceptance probability of at least 95% should be applied. In the case of a sample size of 36 plants, 4 off-types are allowed. For three-way hybrids and open-pollinated varieties, the variability within the variety should not exceed the variability of comparable varieties already known.

*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 further examined by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

4.3.3 Where appropriate, or in cases of doubt, the stability of a hybrid variety may, in addition to an examination of the hybrid variety itself, also be assessed by examination of the uniformity and stability of its parent lines.

## 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: intensity of green color (characteristic 2)
(b) Leaf: blistering (characteristic 3)
(c) Time of beginning of flowering (characteristic 11)
(d) Ray floret: color (characteristic 17)
(e) Disk flower: production of pollen (characteristic 22)
(f) Only inbred lines: Plant: natural height (characteristic 27)
(g) Only hybrids and open-pollinated varieties: Plant: natural height (characteristic 28)
(h) Plant: branching (characteristic 29)
(i) Seed: color (characteristic 39)
(j) Seed: stripes on margin (characteristic 40)
(k) Seed: stripes between margins (characteristic 41)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction and document TGP/9 “Examining Distinctness”.

## 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*

6.2.1 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.

6.2.2 All relevant states of expression are presented in the characteristic.

6.2.3 Further explanation of the presentation of states of expression and notes is provided in document TGP/7 “Development of Test Guidelines”.

*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*

| | English | français | deutsch | español | Example Varieties<br>Exemples<br>Be ejemplo | Note |
|---|---|---|---|---|---|---|
| **1 2** | **3 4** | **5 6** | **7** | | | |
| | **Name of characteristics in English** | **Nom du caractère en français** | **Name des Merkmals auf Deutsch** | **Nombre del carácter en español** | | |
| | states of expression | types d’expression | Ausprägungsstufen | tipos de expresión | | |

1 Characteristic number

2 (*) Asterisked characteristic – see Chapter 6.1.2

3 Type of expression
QL Qualitative characteristic – see Chapter 6.3
QN Quantitative characteristic – see Chapter 6.3
PQ Pseudo-qualitative characteristic – see Chapter 6.3

4 Method of observation (and type of plot, if applicable)
MG, MS, VG, VS – see Chapter 4.1.5

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

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

7 Growth stage key See Explanations on the Table of Characteristics in Chapter 8.3

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

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **1.** | **QN VG** | | | **10** | | | |
| | | **Seedling: anthocyanin coloration of hypocotyl** | | | | | |
| | | absent or very weak | | | | T0954LM | 1 |
| | | weak | | | | OB724 | 2 |
| | | medium | | | | TRC3285 | 3 |
| | | strong | | | | F7AW1MOA | 4 |
| | | very strong | | | | Kisvárdai | 5 |
| **2.** | **(*) QN VG** | | **(a), (b)** | **51-55** | | | |
| | | **Leaf: intensity of green color** | | | | | |
| | | very light | | | | F5DN3MA, T0243HG | 1 |
| | | light | | | | | 2 |
| | | medium | | | | H11050R | 3 |
| | | dark | | | | | 4 |
| | | very dark | | | | 13013 | 5 |
| **3.** | **(*) QN VG** | | **(a), (b)** | **51-55** | | | |
| | | **Leaf: blistering** | | | | | |
| | | absent or very weak | | | | F5DN3MA | 1 |
| | | very weak to weak | | | | | 2 |
| | | weak | | | | F7AX2JA, IR79DMR | 3 |
| | | weak to medium | | | | | 4 |
| | | medium | | | | HA89, IB1088DMR | 5 |
| | | medium to strong | | | | | 6 |
| | | strong | | | | TRC2342 | 7 |
| | | strong to very strong | | | | | 8 |
| | | very strong | | | | | 9 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **4.** | **(*)** | **QN VG** | **(+) (a), (b)** | **51-55** | | | |
| | | **Leaf: serration** | | | | | |
| | | isolated or very fine | | | | 99D40R | 1 |
| | | very fine to fine | | | | | 2 |
| | | fine | | | | IR79DMR | 3 |
| | | fine to medium | | | | | 4 |
| | | medium | | | | HA89, TRC2342 | 5 |
| | | medium to coarse | | | | | 6 |
| | | coarse | | | | PB1458DMR | 7 |
| | | coarse to very coarse | | | | | 8 |
| | | very coarse | | | | | 9 |
| **5.** | | **QN VG** | **(+) (a), (b)** | **53-55** | | | |
| | | **Leaf: shape in cross section** | | | | | |
| | | very concave | | | | RT9513 | 1 |
| | | concave | | | | | 2 |
| | | flat | | | | PH5002R | 3 |
| | | convex | | | | | 4 |
| | | very convex | | | | | 5 |
| **6.** | | **PQ VG** | **(+) (a), (b)** | **53-55** | | | |
| | | **Leaf: shape of distal part** | | | | | |
| | | lanceolate | | | | FR810RM1 | 1 |
| | | lanceolate to narrow triangular | | | | FR81013 | 2 |
| | | narrow triangular | | | | RT0976 | 3 |
| | | medium triangular | | | | RT9513 | 4 |
| | | broad triangular | | | | BT0835 | 5 |
| | | broad triangular to rounded | | | | | 6 |
| | | rounded | | | | | 7 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **7.** | **(*)** | **QN VG** | **(+) (a), (b)** | **53-55** | | | |
| | | **Leaf: auricles** | | | | | |
| | | absent or very small | | | | 37025 | 1 |
| | | very small to small | | | | | 2 |
| | | small | | | | T0954LM | 3 |
| | | small to medium | | | | | 4 |
| | | medium | | | | | 5 |
| | | medium to large | | | | | 6 |
| | | large | | | | F6AH6MO, HA89 | 7 |
| | | large to very large | | | | | 8 |
| | | very large | | | | RHA299 | 9 |
| **8.** | | **QN VG** | **(+) (a), (b)** | **53-55** | | | |
| | | **Leaf: wings** | | | | | |
| | | none or very weakly expressed | | | | T0954LM | 1 |
| | | weakly expressed | | | | F7AW1MOA | 2 |
| | | strongly expressed | | | | 13013 | 3 |
| **9.** | **(*)** | **QN VG** | **(+) (a), (b)** | **53-55** | | | |
| | | **Leaf: angle of lowest lateral veins** | | | | | |
| | | acute | | | | T0860LM | 1 |
| | | right angle or nearly right angle | | | | F7AW1MOA | 2 |
| | | obtuse | | | | TFC3767B | 3 |
| **10** | **(*)** | **QN MS/VG** | **(a), (b)** | **55-57** | | | |
| | | **Leaf: size** | | | | | |
| | | very small | | | | | 1 |
| | | very small to small | | | | | 2 |
| | | small | | | | PH5002R | 3 |
| | | small to medium | | | | | 4 |
| | | medium | | | | LC1093, OB724 | 5 |
| | | medium to large | | | | | 6 |
| | | large | | | | IA1169DMR | 7 |
| | | large to very large | | | | | 8 |
| | | very large | | | | | 9 |

| | | English | | français | | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|---|---|
| **11** | **(*)** | **QN** | **MG/MS** | **(+)** | **(a)** | **61** | | | |
| | | **Time of beginning of flowering** | | | | | | | |
| | | very early | | | | | | PHA283 | 1 |
| | | very early to early | | | | | | | 2 |
| | | early | | | | | | T0860LM | 3 |
| | | early to medium | | | | | | | 4 |
| | | medium | | | | | | H11050R, RHA274 | 5 |
| | | medium to late | | | | | | | 6 |
| | | late | | | | | | RT7710 | 7 |
| | | late to very late | | | | | | | 8 |
| | | very late | | | | | | Kisvárdai, LGR27 | 9 |
| **12** | | **QN** | **VG** | **(+)** | **(a)** | **63-65** | | | |
| | | **Ray floret: attitude of base in relation to head** | | | | | | | |
| | | right angle | | | | | | T0833HG | 1 |
| | | right angle to horizontal | | | | | | | 2 |
| | | horizontal | | | | | | T0954LM | 3 |
| **13** | | **PQ** | **VG** | **(+)** | **(a)** | **63-65** | | | |
| | | **Ray floret: type** | | | | | | | |
| | | flat | | | | | | HA89, IR79DMR | 1 |
| | | longitudinal recurved | | | | | | PH5002R | 2 |
| | | undulated | | | | | | F5DN3MA | 3 |
| | | strongly recurved to back of head | | | | | | | 4 |
| **14** | | **QN** | **VG** | | **(a)** | **63-65** | | | |
| | | **Flower: density of ray florets** | | | | | | | |
| | | very sparse | | | | | | T0954LM | 1 |
| | | sparse | | | | | | | 2 |
| | | medium | | | | | | 99D40R, HA89 | 3 |
| | | dense | | | | | | | 4 |
| | | very dense | | | | | | OB724 | 5 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **15** | | **QN MS/VG** | **(a)** | **63-65** | | | |
| | | **Ray floret: length** | | | | | |
| | | very short | | | | BT0835 | 1 |
| | | short | | | | | 2 |
| | | medium | | | | SF9074MA | 3 |
| | | long | | | | | 4 |
| | | very long | | | | T0954LM | 5 |
| **16** | | **QN MS/VG** | **(+) (a)** | **63-65** | | | |
| | | **Ray floret: width in relation to length** | | | | | |
| | | very narrow | | | | T0954LM | 1 |
| | | moderately narrow | | | | HA850, OB724 | 2 |
| | | moderately broad | | | | | 3 |
| | | very broad | | | | | 4 |
| **17** | **(*)** | **PQ VG** | **(+) (a)** | **63-65** | | | |
| | | **Ray floret: color** | | | | | |
| | | yellowish white | | | | RHA381 | 1 |
| | | light yellow | | | | F7AW1MOA | 2 |
| | | medium yellow | | | | RT7710 | 3 |
| | | orange yellow | | | | U0881BG | 4 |
| | | orange | | | | OB724, P211R | 5 |
| | | purple | | | | | 6 |
| | | reddish brown | | | | | 7 |
| **18** | | **QL VG** | **(a)** | **63-65** | | | |
| | | **Disk flower: anthocyanin coloration of pappi** | | | | | |
| | | absent | | | | F7EW4IMO | 1 |
| | | present | | | | OKD4447R, TRC2342 | 9 |
| **19** | | **PQ VG** | **(a)** | **63-65** | | | |
| | | **Disk flower: color** | | | | | |
| | | yellow | | | | STR226, TRC2342 | 1 |
| | | orange | | | | F7AW1MOA, HA89 | 2 |
| | | purple | | | | | 3 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **20** | | **QL VG** | **(a)** | **63-65** | | | |
| | | **Disk flower: anthocyanin coloration of anther** | | | | | |
| | | absent | | | | R4NO4MJ | 1 |
| | | present | | | | R5XY3MJS | 9 |
| **21** | | **QN VG** | **(+) (a)** | **63-65** | | | |
| | | **Disk flower: anthocyanin coloration of stigma** | | | | | |
| | | absent or very weak | | | | SF9074MA | 1 |
| | | weak | | | | RT7710 | 2 |
| | | medium | | | | R6ST2MI, TRC2342 | 3 |
| | | strong | | | | F7AW1MOA | 4 |
| | | very strong | | | | Kisvárdai | 5 |
| **22** | **(*)** | **QL VG** | **(a)** | **63-65** | | | |
| | | **Disk flower: production of pollen** | | | | | |
| | | absent | | | | F7AW1MOA, HA89 | 1 |
| | | present | | | | IR79DMR, RHA274 | 9 |
| **23** | | **QN VG** | **(+) (a)** | **63-65** | | | |
| | | **Bract: shape** | | | | | |
| | | narrow acute | | | | T0954LM | 1 |
| | | broad acute | | | | IR79DMR | 2 |
| | | rounded | | | | IB1088DMR | 3 |
| **24** | | **QN MS/VG** | **(+) (a)** | **63-65** | | | |
| | | **Bract: length of tip** | | | | | |
| | | very short | | | | IB1088DMR | 1 |
| | | short | | | | | 2 |
| | | medium | | | | HA89, T0954LM | 3 |
| | | long | | | | | 4 |
| | | very long | | | | U0881BG | 5 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **25** | | **QN VG** | **(a)** | **63-65** | | | |
| | | **Bract: intensity of green color of outer side** | | | | | |
| | | light | | | | T0243HG | 1 |
| | | medium | | | | T0954LM | 2 |
| | | dark | | | | RT8711 | 3 |
| **26** | | **QN VG** | **(a)** | **69-73** | | | |
| | | **Bract: attitude in relation to head** | | | | | |
| | | not embracing or very slightly embracing | | | | HA89, RT0976 | 1 |
| | | slightly embracing | | | | F7AW1MOA | 2 |
| | | strongly embracing | | | | RT9513 | 3 |
| **27** | **(*)** | **QN MS** | **(a)** | **69-73** | | | |
| | | **<u>Only inbred lines:</u> Plant: natural height** | | | | | |
| | | very short | | | | FR810RM1 | 1 |
| | | very short to short | | | | | 2 |
| | | short | | | | OB724 | 3 |
| | | short to medium | | | | | 4 |
| | | medium | | | | U0881BG | 5 |
| | | medium to tall | | | | | 6 |
| | | tall | | | | R6ST2MI | 7 |
| | | tall to very tall | | | | | 8 |
| | | very tall | | | | 31G03 | 9 |
| **28** | **(*)** | **QN MS** | **(a)** | **69-73** | | | |
| | | **<u>Only hybrids and open-pollinated varieties:</u> Plant: natural height** | | | | | |
| | | very short | | | | Antonil | 1 |
| | | very short to short | | | | | 2 |
| | | short | | | | GK Milia | 3 |
| | | short to medium | | | | | 4 |
| | | medium | | | | Sumiko | 5 |
| | | medium to tall | | | | | 6 |
| | | tall | | | | Marley | 7 |
| | | tall to very tall | | | | | 8 |
| | | very tall | | | | Kisvárdai | 9 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **29** | **(*)** | **QL VG** | **(a)** | **69-89** | | | |
| | | **Plant: branching** | | | | | |
| | | absent | | | | HA89, OB724 | 1 |
| | | present | | | | RHA274, T0954LM | 9 |
| **30** | **(*)** | **PQ VG** | **(+) (a)** | **69-89** | | | |
| | | **Only varieties with Plant: branching: present: Plant: type of branching** | | | | | |
| | | only basal | | | | | 1 |
| | | predominantly basal | | | | | 2 |
| | | throughout | | | | H11050R | 3 |
| | | predominantly apical | | | | RHA274, T0954LM | 4 |
| | | only apical | | | | TRC2342 | 5 |
| **31** | | **QN VG** | | **69-89** | | | |
| | | **Only varieties with Plant: branching: present: Plant: position of highest lateral head to central head** | | | | | |
| | | below | | | | PH5004R | 1 |
| | | same level | | | | T0954LM | 2 |
| | | above | | | | 99D40R | 3 |
| **32** | **(*)** | **QN VG** | **(+) (a)** | **80-89** | | | |
| | | **Stem: attitude** | | | | | |
| | | straight | | | | U0881BG | 1 |
| | | slightly curved | | | | | 2 |
| | | strongly curved | | | | F7EW2MIA | 3 |
| **33** | **(*)** | **QN VG** | **(+) (a)** | **80-89** | | | |
| | | **Head: attitude** | | | | | |
| | | horizontal | | | | RT8711 | 1 |
| | | inclined | | | | | 2 |
| | | vertical | | | | RT0976 | 3 |
| | | half-turned down | | | | U0881BG | 4 |
| | | turned down | | | | F5DN3MA | 5 |
| | | over turned | | | | | 6 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **34** | **(*)** | **QN MS/VG** | **(a)** | **80-89** | | | |
| | | **Head: size** | | | | | |
| | | very small | | | | | 1 |
| | | very small to small | | | | | 2 |
| | | small | | | | RT0976 | 3 |
| | | small to medium | | | | | 4 |
| | | medium | | | | BT0835, HA89 | 5 |
| | | medium to large | | | | | 6 |
| | | large | | | | F5DN3MA | 7 |
| | | large to very large | | | | | 8 |
| | | very large | | | | | 9 |
| **35** | **(*)** | **PQ VG** | **(+) (a)** | **85-87** | | | |
| | | **Head: shape of grain side** | | | | | |
| | | strongly concave | | | | | 1 |
| | | weakly concave | | | | R5PG6MJ | 2 |
| | | flat | | | | RT8711 | 3 |
| | | weakly convex | | | | HA89, R6ST2MI | 4 |
| | | strongly convex | | | | T0916LG | 5 |
| | | deformed | | | | TRC3398R | 6 |
| **36** | | **QN MS/VG** | **(a)** | **99** | | | |
| | | **Seed: size** | | | | | |
| | | very small | | | | PHA283 | 1 |
| | | very small to small | | | | | 2 |
| | | small | | | | TRC2342 | 3 |
| | | small to medium | | | | | 4 |
| | | medium | | | | HA89, OB724 | 5 |
| | | medium to large | | | | | 6 |
| | | large | | | | FT2603, Kisvárdai | 7 |
| | | large to very large | | | | | 8 |
| | | very large | | | | | 9 |
| **37** | **(*)** | **PQ VG** | **(+) (a)** | **99** | | | |
| | | **Seed: shape** | | | | | |
| | | elongated | | | | BT0835 | 1 |
| | | narrow ovoid | | | | H11050R | 2 |
| | | broad ovoid | | | | F7AW1MOA, HA89 | 3 |
| | | rounded | | | | | 4 |

| | | English | français | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|
| **38** | | **QN MS/VG** | **(a)** | **99** | | | |
| | | **Seed: thickness relative to width** | | | | | |
| | | very thin | | | | RHA801 | 1 |
| | | thin | | | | | 2 |
| | | medium | | | | F7AW1MOA, FR83322 | 3 |
| | | thick | | | | 85C11R, F7AX2MA | 4 |
| | | very thick | | | | | 5 |
| **39** | **(*)** | **PQ VG** | **(+) (a)** | **99** | | | |
| | | **Seed: color** | | | | | |
| | | white | | | | Labud | 1 |
| | | purple | | | | | 2 |
| | | light brown | | | | IR79DMR | 3 |
| | | medium brown | | | | H11050R | 4 |
| | | dark brown | | | | B0644LM | 5 |
| | | light grey | | | | RW666IMI | 6 |
| | | medium grey | | | | RT9513 | 7 |
| | | dark grey | | | | | 8 |
| | | black | | | | HA89, T0954LM | 9 |
| **40** | **(*)** | **QN VG** | **(+) (a)** | **99** | | | |
| | | **Seed: stripes on margin** | | | | | |
| | | none or very weakly expressed | | | | T0954LM | 1 |
| | | weakly expressed | | | | OB724 | 2 |
| | | strongly expressed | | | | HA89, U0881BG | 3 |

| | | English | | français | | deutsch | español | Example Varieties<br>Exemples<br>Beispielssorten<br>Variedades ejemplo | Note/<br>Nota |
|---|---|---|---|---|---|---|---|---|---|
| **41** | **(*)** | **QN** | **VG** | **(+)** | **(a)** | **99** | | | |
| | | **Seed: stripes between margins** | | | | | | | |
| | | none or very weakly expressed | | | | | | T0954LM | 1 |
| | | weakly expressed | | | | | | LGR27 | 2 |
| | | strongly expressed | | | | | | HA89, U0881BG | 3 |
| **42** | **(*)** | **PQ** | **VG** | | **(a)** | **99** | | | |
| | | **Seed: color of stripes** | | | | | | | |
| | | white | | | | | | U0881BG | 1 |
| | | grey | | | | | | 99D40R | 2 |
| | | brown | | | | | | F1164LM | 3 |
| | | black | | | | | | | 4 |

8. Explanations on the Table of Characteristics

*8.1 Explanations covering several characteristics*

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

(a) Observations should be made on the main stem.

(b) Observations on the leaf should be made on fully developed leaves at the 2/3 height of the plant.

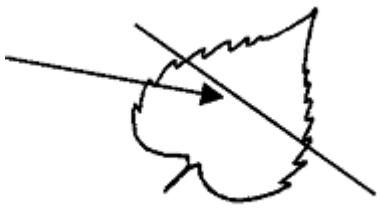
*8.2 Explanations for individual characteristics*

Ad. 4: Leaf: serration

| 1 | 3 | 5 | 7 | 9 |
|---|---|---|---|---|
| isolated or very fine | fine | medium | coarse | very coarse |

Ad. 5: Leaf: shape in cross section

Cross section:



| 1 | 3 | 5 |
|---|---|---|
| very concave | flat | very convex |

Ad. 6: Leaf: shape of distal part

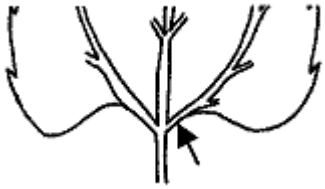
Observations should be made on the upper two-thirds.

Ad. 7: Leaf: auricles

| 1 | 3 | 5 | 7 | 9 |
|---|---|---|---|---|
| absent or very small | small | medium | large | very large |

Ad. 8: Leaf: wings

(Parenchyma at base of lateral veins)



1
none or very weakly expressed

2
weakly expressed

3
strongly expressed

Ad. 9: Leaf: angle of lowest lateral veins

1
acute

2
right angle or nearly right angle

3
obtuse

Ad. 11: Time of beginning of flowering

Time of flowering is reached when 50% of the plants have at least one extended ray floret.

Ad. 12: Ray floret: attitude of base in relation to head

1
right angle

3
horizontal

Ad. 13: Ray floret: type

1
flat

2
longitudinal recurved

3
undulated

4
strongly recurved to back of head

Ad. 16: Ray floret: width in relation to length

1
very narrow

2
moderately narrow

3
moderately broad

4
very broad

Ad. 17: Ray floret: color

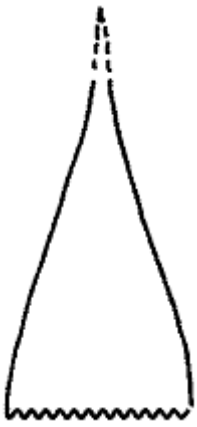
If more than one color, only the color covering the largest area is considered.

Ad. 21: Disk flower: anthocyanin coloration of stigma

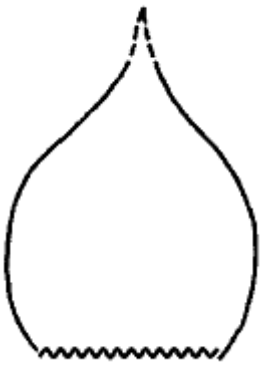
Observation should be made on the stigma just after the pollen appears at the top of the anthers.

Ad. 23: Bract: shape

1
narrow

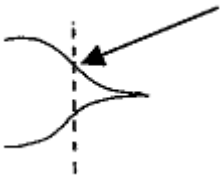


2
intermediate



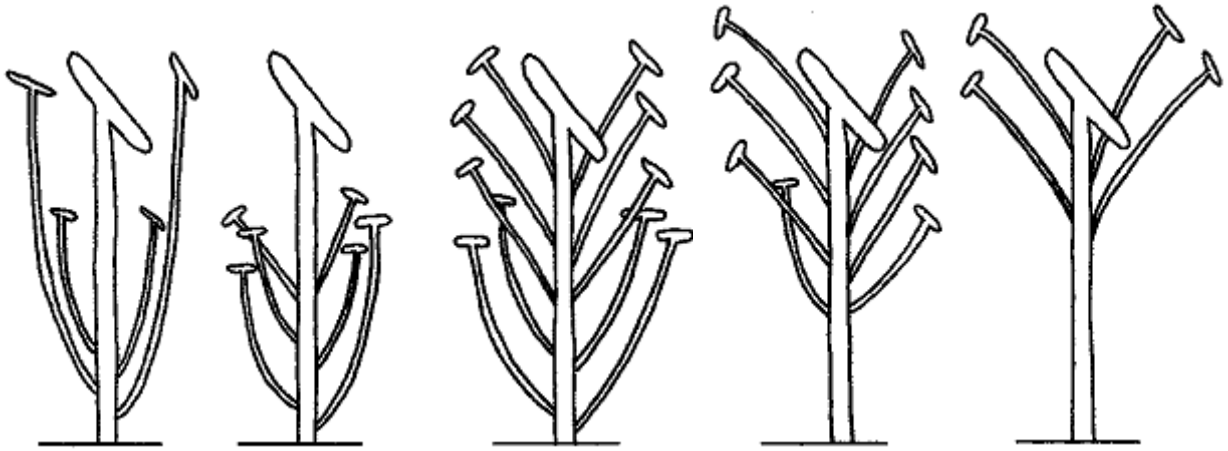
3
broad

Ad. 24: Bract: length of tip



Tip begins where the direction of curving changes

Ad. 30: Only varieties with Plant: branching: present: Plant: type of branching

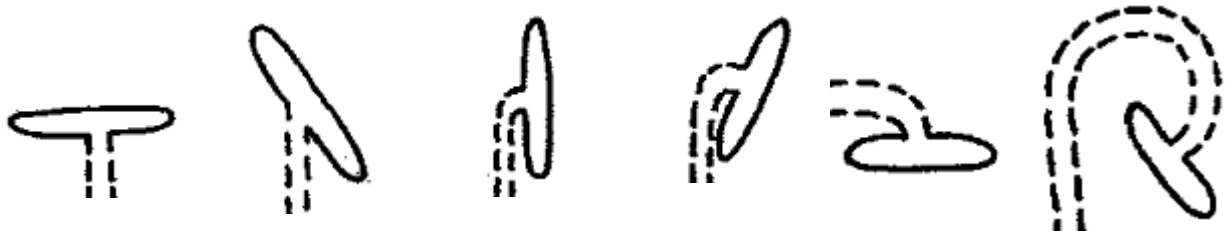


| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| only basal | predominantly basal | overall | predominantly apical | only apical |

Ad. 32: Stem: attitude

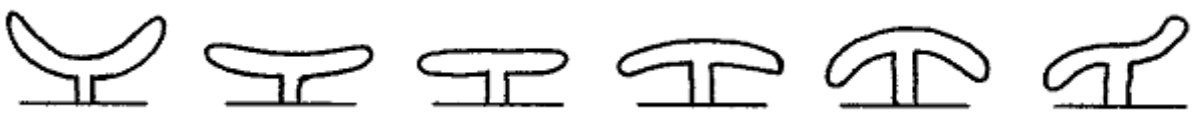
To be observed on the 1/3 upper part of the stem under the head.

Ad. 33: Head: attitude



| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| horizontal | inclined | vertical | half-turned down | turned down | over turned |

Ad. 35: Head: shape of grain side



| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| strongly concave | weakly concave | flat | weakly convex | strongly convex | deformed |

Ad. 37: Seed: shape

1
elongated

2
narrow ovoid

3
broad ovoid

4
rounded

Ad. 39: Seed: color

The color with the largest surface area should be observed. In cases where the areas of the colors are too similar to reliably decide which color has the largest area, the darker color is to be observed.

Ad. 40: Seed: stripes on margin

Ad. 41: Seed: stripes between margins

*8.3 Growth stage of Helianthus annuus L. adopted to the BBCH (Meier U., 1997) scale applicable to individual plant*

Code Description

**Principal growth stage 0: Germination**
00 Dry seed (achene)
01 Beginning of seed imbibition
03 Seed imbibition complete
05 Radicle emerged from seed
06 Radicle elongated, root hairs developing
07 Hypocotyl with cotyledons emerged from seed
08 Hypocotyl with cotyledons growing towards soil surface
09 Emergence: cotyledons emerge through soil surface
**Principal growth stage 1: Leaf development1**
10 Cotyledons completely unfolded
12 2 leaves (first pair) unfolded
14 4 leaves (second pair) unfolded
15 5 leaves unfolded
16 6 leaves unfolded
17 7 leaves unfolded
18 8 leaves unfolded
19 9 or more leaves unfolded
(Stem elongation may occur earlier than stage 19; in this case continue with the principal stage 3)
**Principal growth stage 3: Stem elongation**
30 Beginning of stem elongation
31 1 visibly extended internode
32 2 visibly extended internodes
33 3 visibly extended internodes
3 . Stages continuous till . . .
39 9 or more visibly extended internodes
**Principal growth stage 5: Inflorescence emergence**
51 Inflorescence just visible between youngest leaves
53 Inflorescence separating from youngest leaves, bracts distinguishable from foliage leaves
55 Inflorescence separated from youngest foliage leaf
57 Inflorescence clearly separated from foliage leaves
59 Ray florets visible between the bracts; inflorescence still closed
**Principal growth stage 6: Flowering**
61 Beginning of flowering: ray florets extended, disc florets visible in outer third of inflorescence
63 Disc florets in outer third of inflorescence in bloom (stamens and stigmata visible)
65 Full flowering: disc florets in middle third of inflorescence in bloom (stames and stigmata visible)
67 Flowering declining: disc florets in inner third of inflorescence in bloom (stames and stigmata visible)
69 End of flowering: most disc florets have finished flowering, ray florets dry or fallen
**Principal growth stage 7: Development of fruit**
71 Seeds on outer edge of the inflorescence are grey and have reached final size
73 Seeds on outer third of the inflorescence are grey and have reached final size
75 Seeds on middle third of the inflorescence are grey and have reached final size
79 Seeds on inner third of the inflorescence are grey and have reached final size
**Principal growth stage 8: Ripening**
80 Beginning of ripening: seeds on outer third of anthocarp black and hard. Back of anthocarp still green
81 Seeds on outer third of anthocarp dark and hard. Back ofanthocarp still green
83 Dark of anthocarp yellowish-green, bracts still green. Seeds about 50% dry matter
85 Seeds on middle third of anthocarp dark and hard. Back of anthocarp yellow, bracts brown edged. Seeds about 60% dry matter
87 Physiological ripeness: back of the anthocarp yellow. Bracts marbled brown. Seeds about 75–80% dry matter
89 Fully ripe: seeds on inner third of anthocarp dark and hard. Back of anthocarp brown. Bracts brown. Seeds about 85% dry matter
**Principal growth stage 9:**
92 Over ripe, seeds over 90% dry matter
97 Plant dead and dry
99 Harvested product

## 9. Literature

ASFIS, GEVES, GNIS: “Description des géniteurs et variétés de tournesol”, édition 2000 (English, French, Spanish) ASFIS, 44, rue du Louvre, 75001 Paris, FR

Meier U., 1997: Growth stages of mono- and dicotyledonous plants: BBCH-Monograph. Wien Federal Biological Research Center for Agriculture and Forestry, Blackwell Wissenschafts-Verlag, Berlin, DE.

Miller J.F.: “Update on Inheritance of Sunflower Characteristics,” USDA - ARS, Northern Crop Science Laboratory, Fargo, North Dakoto 58105, USA

10. Technical Questionnaire

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

| | Application date:<br>(not to be filled in by the applicant) |
|---|---|

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

In the case of hybrid varieties which are the subject of an application for plant breeders' rights, and where the parent lines are to be submitted as a part of the examination of the hybrid variety, this Technical Questionnaire should be completed for each of the parent lines, in addition to being completed for the hybrid variety.

1. Subject of the Technical Questionnaire

| | | |
|---|---|---|
| 1.1 | Botanical name | *Helianthus annuus* L. |
| 1.2 | Common name | Common Sunflower |

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

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]

(please state parent variety)

(……..……..…) x (……..……..…)

female parent male parent

(b) partially known cross [ ]

(please state known parent variety(ies))

(……..……..…) x (……..……..…)

female parent male parent

(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)

| |
|---|
| |

# 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: |
|---|---|---|

4.2 Method of propagating the variety

4.2.1 Seed-propagated varieties

| | | |
|---|---|---|
| (a) | Cross-pollination | [ ] |
| (i) | Population | [ ] |
| (i) | Single hybrid | [ ] |
| (b) | Hybrid | [ ] |
| (ii) | Three-way hybrid | [ ] |
| (iii) | Male sterile hybrid | [ ] |
| (iv) | Male fertile hybrid | [ ] |
| (c) | Inbred line | [ ] |
| (i) | Male sterile line | [ ] |
| (ii) | Male fertile line | [ ] |
| (d) | Other (please provide details) | [ ] |

4.2.2 Other [ ]
(Please provide details)

In the case of hybrid varieties the production scheme for the hybrid should be provided on a separate sheet. This should provide details of all the parent lines required for propagating the hybrid e.g.

Single Hybrid

(……..……..…) x (……..……..…)

female parent male parent

Three-Way Hybrid

(……..……..…) x (……..……..…)

female line male line

(……..……..…) x (……..……..…)

single hybrid used as female parent male parent

and should identify in particular:

(a) any male sterile lines

(b) In case of use of the male sterility system, indicate the name of the maintainer line of the female parental line.

| 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: intensity of green color** | | |
| | very light | F5DN3MA, T0243HG | 1 [ ] |
| | light | | 2 [ ] |
| | medium | H11050R | 3 [ ] |
| | dark | | 4 [ ] |
| | very dark | 13013 | 5 [ ] |
| **5.2 (3)** | **Leaf: blistering** | | |
| | absent or very weak | F5DN3MA | 1 [ ] |
| | very weak to weak | | 2 [ ] |
| | weak | F7AX2JA, IR79DMR | 3 [ ] |
| | weak to medium | | 4 [ ] |
| | medium | HA89, IB1088DMR | 5 [ ] |
| | medium to strong | | 6 [ ] |
| | strong | TRC2342 | 7 [ ] |
| | strong to very strong | | 8 [ ] |
| | very strong | | 9 [ ] |
| **5.3 (11)** | **Time of beginning of flowering** | | |
| | very early | PHA283 | 1 [ ] |
| | very early to early | | 2 [ ] |
| | early | T0860LM | 3 [ ] |
| | early to medium | | 4 [ ] |
| | medium | H11050R, RHA274 | 5 [ ] |
| | medium to late | | 6 [ ] |
| | late | RT7710 | 7 [ ] |
| | late to very late | | 8 [ ] |
| | very late | Kisvárdai, LGR27 | 9 [ ] |

| | Characteristics | Example Varieties | Note |
|---|---|---|---|
| **5.4 (17)** | **Ray floret: color** | | |
| | yellowish white | RHA381 | 1 [ ] |
| | light yellow | F7AW1MOA | 2 [ ] |
| | medium yellow | RT7710 | 3 [ ] |
| | orange yellow | U0881BG | 4 [ ] |
| | orange | OB724, P211R | 5 [ ] |
| | purple | | 6 [ ] |
| | reddish brown | | 7 [ ] |
| **5.5 (22)** | **Disk flower: production of pollen** | | |
| | absent | F7AW1MOA, HA89 | 1 [ ] |
| | present | IR79DMR, RHA274 | 9 [ ] |
| **5.6 (27)** | **Only inbred lines: Plant: natural height** | | |
| | very short | FR810RM1 | 1 [ ] |
| | very short to short | | 2 [ ] |
| | short | OB724 | 3 [ ] |
| | short to medium | | 4 [ ] |
| | medium | U0881BG | 5 [ ] |
| | medium to tall | | 6 [ ] |
| | tall | R6ST2MI | 7 [ ] |
| | tall to very tall | | 8 [ ] |
| | very tall | 31G03 | 9 [ ] |
| **5.7 (28)** | **Only hybrids and open-pollinated varieties: Plant: natural height** | | |
| | very short | Antonil | 1 [ ] |
| | very short to short | | 2 [ ] |
| | short | GK Milia | 3 [ ] |
| | short to medium | | 4 [ ] |
| | medium | Sumiko | 5 [ ] |
| | medium to tall | | 6 [ ] |
| | tall | Marley | 7 [ ] |
| | tall to very tall | | 8 [ ] |
| | very tall | Kisvárdai | 9 [ ] |
| **5.8 (29)** | **Plant: branching** | | |
| | absent | HA89, OB724 | 1 [ ] |
| | present | RHA274, T0954LM | 9 [ ] |

| | Characteristics | Example Varieties | Note |
|---|---|---|---|
| **5.9 (39)** | **Seed: color** | | |
| | white | Labud | 1 [ ] |
| | purple | | 2 [ ] |
| | light brown | IR79DMR | 3 [ ] |
| | medium brown | H11050R | 4 [ ] |
| | dark brown | B0644LM | 5 [ ] |
| | light grey | RW666IMI | 6 [ ] |
| | medium grey | RT9513 | 7 [ ] |
| | dark grey | | 8 [ ] |
| | black | HA89, T0954LM | 9 [ ] |
| **5.10 (40)** | **Seed: stripes on margin** | | |
| | none or very weakly expressed | T0954LM | 1 [ ] |
| | weakly expressed | OB724 | 2 [ ] |
| | strongly expressed | HA89, U0881BG | 3 [ ] |
| **5.11 (41)** | **Seed: stripes between margins** | | |
| | none or very weakly expressed | T0954LM | 1 [ ] |
| | weakly expressed | LGR27 | 2 [ ] |
| | strongly expressed | HA89, U0881BG | 3 [ ] |

| 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* | *Time of beginning of flowering* | *early (3)* | *late (7)* |
| | | | |
| | | | |
| | | | |

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

(1) Use
(a) oil and cake
(b) birds consumption
(c) direct human consumption (hulling type)
(d) direct human consumption (confectionary)
(e) other use (please specify)

(2) Resistance to pests nad diseases
(a) Downy mildew (precise the races)
(b) Broomrape (precise the races)
(c) other pests or diseases (please specify)

(3) Oleic acid content
(a) low
(b) medium
(c) high

(4) Tolerance to herbicides
(a) yes (please specify)
(b) no

# 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]

ANNEX

## Part I
## Introduction

The following Annex contains a list of characteristics derived by using electrophoresis and a description of the method to be used. UPOV decided to place these characteristics in an Annex to the Test Guidelines, thereby creating a special category of characteristic, because the majority of the UPOV member States is of the view that it is not possible to establish distinctness solely on the basis of a difference found in a characteristic derived by using electrophoresis. Such characteristics should therefore only be used as a complement to other differences in morphological or physiological characteristics. UPOV reconfirms that these characteristics are considered useful but that they might not be sufficient on their own to establish distinctness. They should not be used as a routine characteristic but at the request or with the agreement of the applicant of the candidate variety.

## Part II
## Characteristics Derived by Using Electrophoresis

| Nr. | Characteristic | States of expression | Example varieties | Note |
|---|---|---|---|---|
| 42 | **Allele expression at locus Me1** | Genotype 2/2 | IB1088DMR | 1 |
| | | Genotype 4/4 | SF9074MA | 2 |
| | | Genotype 2/4 | Sumiko | 3 |
| 43 | **Allele expression at locus Pgd1** | Genotype 2/2 | IB1088DMR | 1 |
| | | Genotype 4/4 | SF9074MA | 2 |
| | | Genotype 2/4 | Sumiko | 3 |
| 44 | **Allele expression at locus Pgi2** | Genotype 2/2 | IB1088DMR | 1 |
| | | Genotype 4/4 | SF9074MA | 2 |
| | | Genotype 2/4 | GK Petrus CLP | 3 |
| 45 | **Allele expression at locus Shdh1** | Genotype 2/2 | IB1088DMR | 1 |
| | | Genotype 4/4 | | 2 |
| | | Genotype 2/4 | Marley | 3 |
| 46 | **Allele expression at locus Pgm4** | Genotype 2/2 | | 1 |
| | | Genotype 4/4 | IB1088DMR | 2 |
| | | Genotype 2/4 | GK Petrus CLP | 3 |

## Part III

## Description of the Method to be Used

## Description of the SGE Method for the Analysis of Isoenzymes from *Helianthus annuus L.*

**1. Number of seedlings per test :**

• For checking formula:
10 seedlings each of inbred lines
4 seedlings of single hybrids
10 seedlings of three-way hybrids

• For distinctness, uniformity and stability test:
at least 40 seedlings for inbred lines, hybrids and open-pollinated varieties

If enzyme electrophoresis is used for testing distinctness, the same population standard and the same acceptance probability as for other characteristics should be applied. All plants within an inbred line with one locus or more loci being heterozygous with one allele in each locus coming from the inbred line (e.g. AX) should be considered out-crosses. All other cases of heterozygosity as well as cases where one foreign allele is present in one locus with homozygous status should be considered off-types.

**2. Apparatus and equipment**

Any suitable horizontal electrophoresis system can be used, provided that the gels can be kept at 4° C. A gel thickness of 10 mm is recommended. The power supply used should be capable of delivering constant voltage output.

3. **Chemicals**

All chemicals should be of 'Analytical Reagent' grade or better.

3.1 Chemicals for enzyme extraction:
Tris- (hydroxymethyl) aminomethane (Tris)
Hydrochloric acid
β-Mercaptoethanol

3.2 Chemicals for electrophoresis
Bromophenol blue
Citric acid monohydrate
L-Histidine
Starch hydrolysed, for electrophoresis, (Sigma S-4501 or equivalent)

3.3 Chemicals for staining enzymes
95% Ethanol
Ethylenediamine tetra-acetic acid, disodium salt (EDTA Na2)
D-Fructose 6-phosphate, disodium salt
α-D-Glucose 1-phosphate, monohydrate, disodium salt
Glucose 6-phosphate dehydrogenase (Sigma G5885)
Hydrochloric acid (HCl)
Magnesium chloride hexahydrate (MgCl2, 6H2O)
DL-Malic acid, monosodium salt
Dimethylthiazol diphenyl tetrazolium (MTT)
β-Nicotinamide adenine dinucleotide phosphate (NADP)
Nitro-blue tetrazolium (NBT)
6-phosphogluconic acid, trisodium salt dihydrate
Phenazine methosulfate (PMS)
Shikimic acid
Sodium hydroxide (NaOH)
Tris- (hydroxymethyl) aminomethane (Tris)

4. **Solutions**

4.1 Extraction solution: 0.1M Tris HCl (pH 7.2) + 0.2 % 2-mercaptoethanol (v/v).

4.2. Electrophoresis buffers

4.2.1 Buffers for SGE pH 6.5

4.2.1.1 Stock solution: 0.364 M L-histidine-citrate

50.44 g L-histidine
8.34 g Citric acid monohydrate
made up to 1 l with de-ionised water

4.2.1.2 Running buffer: 0.072 M L-histidine-citrate pH 6.5 (Stock solution diluted 1 in 5)

400 ml stock solution (4.2.1.1)
made up to 2 l with de-ionised water

4.2.1.3 Gel buffer: 0.024 M L-histidine-citrate (Stock solution diluted 1 in 15)

80 ml stock solution (4.2.1.1)
made up to 1200 ml with de-ionised water

4.2.2 Buffers for SGE pH 5.7

4.2.2.1 Running buffer: 0.067 M L-histidine-citrate pH 5.7:

20.18 g L-histidine
8.34 g Citric acid monohydrate
made up to 2 l with de-ionised water

4.2.2.2 Gel buffer: 0.011 M L-histidine-citrate (Running buffer diluted 1 in 6):

100 ml running buffer (4.2.2.1) made up to 1200 ml with de-ionised water

4.2.2.3 Bromophenol blue solution:

50 mg bromophenol blue dissolved in 100 ml de-ionised water

4.3 Staining solutions

4.3.1 Stock solutions

4.3.1.1 1 M Tris-HCl pH 7.5

121.1 g Tris, made up to 1 l with de-ionised water and adjusted to pH 7.5 with 50 % HCl

4.3.1.2 1 M Tris-HCl pH 8.5

121.1 g Tris, made up to 1 l with de-ionised water and adjusted to pH 8.5 with 50 % HCl

4.3.1.3 MTT solution

1.0 g MTT made up to 100 ml with de-ionised water

4.3.1.4 NBT solution

1.0 g NBT made up to 100 ml with de-ionised water

4.3.1.5 PMS solution

200 mg PMS made up to 100 ml with de-ionised water

4.3.1.6 MgCl2 solution

10 g Magnesium chloride hexahydrate made up to 100 ml with de-ionised water

4.3.1.7 Sodium malate solution

2.5 g DL-malic acid
made up to 50 ml with de-ionised water and adjusted to pH 8.0 with 1M NaOH.

4.3.2 Staining solutions

4.3.2.1 ME staining solution

100 ml 0.1 M Tris HCl, pH 7.5 (4.3.1.1 diluted 1 in 10)
4 ml Sodium malate solution (4.3.1.7.)
1 ml NBT solution (4.3.1.4.)
1 ml PMS solution (4.3.1.5.)
1,8 ml MgCl2 solution (4.3.1.6.)
17.5 mg NADP

4.3.2.2 PGD + PGI staining solution

100 ml 0.1 M Tris HCl, pH 7.5 (4.3.1.1. diluted 1 in 10)
100 mg D-Fructose 6-phosphate Na2 salt
60 mg 6-Phosphogluconic acid Na3 salt
10 mg NADP
1 ml MTT solution (4.3.1.3.)
1.5 ml PMS solution (4.3.1.5.)
1 ml MgCl2 solution (4.3.1.6.)
40 units of Glucose-6-phosphate dehydrogenase (SIGMA G 5885)
To stain PGI only, do not include 6-phosphogluconic acid.
To stain PGD only, do not include either fructose 6-phosphate disodium salt or glucose 6-phosphate dehydrogenase.

4.3.2.3 ShDH staining solution

100 ml 0.2 M Tris HCl, pH 8.5 (4.3.1.2 diluted 1 in 5)
50 mg shikimic acid
1 ml MTT solution (4.3.1.3)
1.25 ml PMS solution (4.3.1.5)
12 mg NADP

4.3.2.4 PGM staining solution

100 ml 0.1 M Tris HCl, pH 8.5 (4.3.1.2. diluted 1 in 10)
150 mg α-D-Glucose 1-phosphate 1H2O, Na2 salt
150 mg EDTA, Na2
10 mg NADP
1.5 ml MTT solution (4.3.1.3)
1.ml PMS solution (4.3.1.5)
4 ml MgCl2 solution (4.3.1.6)
40 units of Glucose 6-phosphate dehydrogenase

5. **Procedure**

5.1. Enzyme extraction

Seedlings are grown on moistened germination paper, at 25°C, in darkness, for 2 to 3 days. Seed coats are removed and cotyledons are crushed at 4°C, with a pestle in 1.5 ml microtubes containing 300 µl extraction buffer (4.1). The extracts can be stored at -30°C or at -80°C.

5.2 Preparation of the gel

Prepare the gels the day before migration. To make two 12.5 % starch gels (18 x 18 x 1 cm) the following is required: 128 g starch are mixed in 1020 ml gel buffer (4.2.1.3 or 4.2.2.2) in a 1000 ml Büchner flask and heated at 78°C. The mixture is degassed with a water jet aspirator for 30 seconds. The gels are poured into gel moulds as described in the user's manual of the equipment used. The formation of air bubbles should be avoided. The gels are allowed to cool at room temperature for 45 min, then placed in a refrigerator for 1 h. The gels are wrapped with polyethylene film for overnight storage. and cooled to 4°C for 1 h before migration.

5.3 Electrophoresis

5.3.1 Each electrode tank is filled with the appropriate volume of running buffer (4.2.1.2 or 4.2.2.1) pre-cooled to 4°C. The polyethylene film is lifted up and two transversal slits are cut in the gel 3 cm and 4 cm from the edge (cathode side) of the mould.

The 1 cm gel slice is removed and the extracts are loaded as follows:

The enzyme extracts are thawed from 5.1, and absorbed on a filter paper wick (1.5 mm x 20 mm, Whatman N° 3).
The wicks are inserted into the gel, tightly against the first slit. One wick soaked with bromophenol blue

solution (4.2.2.3) (migration dye marker) is placed on each side of the gel.
The gel slice is cautiously replaced. Each gel is covered with polyethylene film.

The two gels, with the extracts on the cathodal side, are placed on the two electrode buffer tanks, in a refrigerated cabinet at 4°C.
The electrophoresis is carried out at 4°C, towards the anode. After 15 min of migration at the first voltage, the wicks are removed and the voltage is increased. Constant voltage should be maintained during each phase.

The electrophoretic conditions are indicated in the following table.

| Buffer systems | Constant voltage | Distance run by bromophenol blue | Duration of migration |
|---|---|---|---|
| Histidine citrate pH 5.7 | 260 V for 15 min then 290 V | 13 cm | 5 h |
| Histidine citrate pH 6.5 | 240 V for 15 min then 280 V | 11 cm | 5 h |

SGE at pH 5.7 should be used for detecting ME, PGD and PGI. The isoenzymes PGM and ShDH should be analysed by SGE pH 6.5.

5.4 Enzyme staining

After switching off the current, the gel is cut horizontally in 1 mm thick slices with a very fine steel wire or a fishing line. The upper slice is discarded. Individual gel slices are stained by incubation at 37°C, in darkness in the following solutions:

for ME: solution 4.3.2.1, incubation time: 15 h
for PGD and PGI: solution 4.3.2.2, incubation time: 1 h
for SHDH: solution 4.3.2.3, incubation time: 1 h
for PGM: solution 4.3.2.4, incubation time: 1/2 h

After staining the gel slices are rinsed in de-ionised water and fixed in 40% ethanol solution. The following procedures for long time storing can be successfully used: e.g. drying of the gels between two cellophane sheets soaked in a 5% glycerol solution, or storing in sealed polyethylene bags.

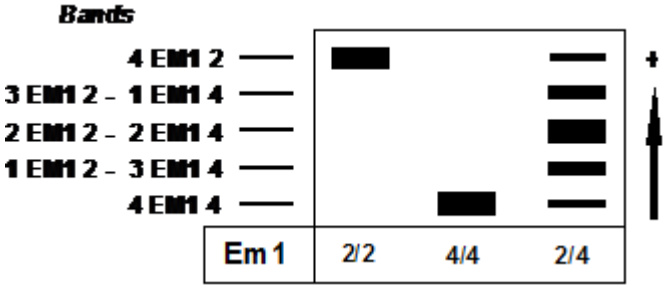
6. **Recognition of the alleles encoding isoenzymes**

6.1 Recognition of the alleles encoding ME

6.1.1 Genetic interpretation of the zymogrammes

| Enzyme | Quaternary structure | Locus | Alleles |
|---|---|---|---|
| Malic enzyme (ME) | Tetrameric | Me1 | 2<br>4 |

6.1.2 Schematization of the zymogrammes

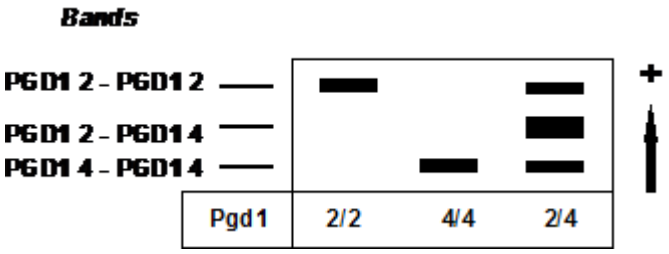


6.2 Recognition of the alleles encoding PGD

6.2.1 Genetic interpretation of the zymogrammes

| Enzyme | Quaternary structure | Locus | Alleles |
|---|---|---|---|
| 6-phosphogluconate dehydrogenase (PGD) | Dimeric | Pgd1 | 2<br>4 |

6.2.2 Schematization of the zymogrammes



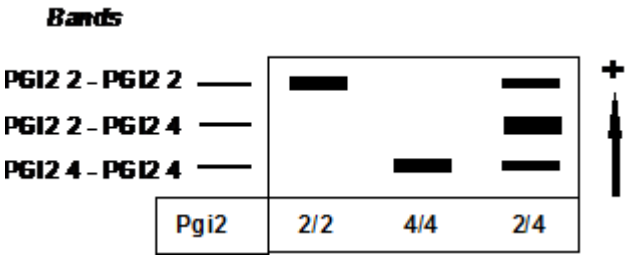

Two migration zones can be observed; only the slowest migrating bands are polymorphic.

6.3 Recognition of the alleles encoding PGI

6.3.1 Genetic interpretation of the zymogrammes

| Enzyme | Quaternary structure | Locus | Alleles |
|---|---|---|---|
| Phosphoglucoisomerase (PGI) | Dimeric | Pgi2 | 2<br>4 |

6.3.2 Schematization of the zymogrammes



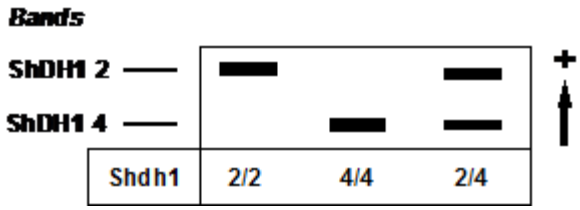

There are two migration zones; only the slowest migrating bands are scored.

6.4 Recognition of the alleles encoding ShDH

6.4.1 Genetic interpretation of the zymogrammes

| Enzyme | Quaternary structure | Locus | Alleles |
|---|---|---|---|
| Shikimate dehydrogenase (ShDH) | Monomeric | Shdh1 | 2<br>4 |

6.4.2 Schematization of the zymogrammes

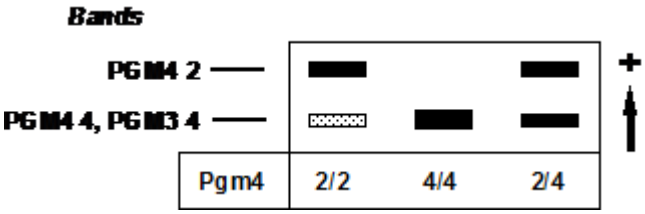


6.5 Recognition of the alleles encoding PGM

6.5.1 Genetic interpretation of the zymogrammes

| Enzyme | Quaternary structure | Locus | Alleles |
|---|---|---|---|
| Phosphoglucomutase (PGM) | Monomeric | Pgm4 | 2<br>4 |

6.5.2 Schematization of the zymogrammes




Several migration zones can be observed; only the fastest zone is polymorphic.

There is another gene which has not been considered. This has been designated Pgm3, encoding an enzyme which comigrates with PGM4 4.

So, the genotypes Pgm4 2/2 and Pgm4 2/4 give a two-band zymogramme. These both genotypes differ only by relative band intensities.

[End of Annex and of document]