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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

COMMON 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 forty-seventh session, to be held in Naivasha, Kenya,
from 2018-05-21 to 2018-05-25

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.]

TABLE OF CONTENTS PAGE

1. Subject of these Test Guidelines

1.1 These Test Guidelines apply to all varieties of *Helianthus annuus* L..

1.2 In the case of ornamental varieties, in particular, it may be necessary to use additional characteristics or additional states of expression to those included in the Table of Characteristics in order to examine Distinctness, Uniformity and Stability.

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 seeds for inbred lines
0.5 kg for hybrids and open-pollinated varieties
In the case of hybrid varieties, an additional 0.5 kg 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 0.5 kg 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.

3.2 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 40 plants or parts of plants taken from each of 40 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-propogated 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 varieties, a population standard of 2% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 40 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 40 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.

4.2.7 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.

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 13)
(d) Ray floret: color (characteristic 19)
(e) Disk flower: pro-duction of pollen (characteristic 22)
(f) Plant: natural height (characteristic 26)
(g) Plant: branching (characteristic 27)
(h) Seed: main color (characteristic 37)
(i) Seed: stripes on margin (characteristic 38)
(j) Seed: stripes be-tween margins (characteristic 39)

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 In the case of qualitative and pseudo-qualitative characteristics (see Chapter 6.3), all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines may be abbreviated as follows:

State	*Note*
small	3
medium	5
large	7

However, it should be noted that all of the following 9 states of expression exist to describe varieties and should be used as appropriate:

State	*Note*
very small	1
very small to small	2
small	3
small to medium	4
medium	5
medium to large	6
large	7
large to very large	8
very large	9

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 Exemples Beispielssorten Variedades ejemplo	Note/ Nota
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

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

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1.		**QN**	**VG**			**10**			
		Seedling: anthocyanin coloration of hypocotyl							
		absent or very weak							1
		weak							2
		medium							3
		strong							4
		very strong							5
2.	**(*)**	**QN**	**VG**		**(a), (b)**	**51-55**			
		Leaf: intensity of green color							
		weak							3
		medium							5
		strong							7
3.	**(*)**	**QN**	**VG**		**(a), (b)**	**51-55**			
		Leaf: blistering							
		absent or very weak							1
		weak							3
		medium							5
		strong							7
		very strong							9
4.	**(*)**	**QN**	**VG**	**(+)**	**(a), (b)**	**51-55**			
		Leaf: serration							
		isolated or very fine							1
		fine							3
		medium							5
		coarse							7
		very coarse							9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
5.		**QL VG**	**(+) (a), (b)**	**51-55**			
		Leaf: shape of cross section					
		strongly concave					1
		weakly concave					2
		flat					3
		weakly convex					4
		strongly convex					5
6.		**PQ VG**	**(+) (a), (b)**	**51-55**			
		Leaf: shape of distal part					
		lanceolate					1
		lanceolate to narrow triangular					2
		narrow triangular					3
		narrow triangular to broad triangular					4
		broad triangular					5
		broad triangular to acuminate					6
		broad triangular to rounded					7
		acuminate					8
		rounded					9
7.	**(*)**	**QN VG**	**(+) (a), (b)**	**51-55**			
		Leaf: auricules					
		none or very small					1
		small					3
		medium					5
		large					7
		very large					9
8.		**QL VG**	**(+) (a), (b)**	**51-55**			
		Leaf: wings					
		none or very weakly expressed					1
		weakly expressed					2
		strongly expressed					3

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
9.	**(*)** **QN** **VG**		**(+)** **(a), (b)**	**51-55**			
		Leaf: angle of lowest lateral veins					
		acute					1
		right angle or nearly right angle					2
		obtuse					3
10.	**QN** **VG**		**(+)** **(a), (b)**	**51-55**			
		Leaf: height of the tip of the blade compared to insertion of petiole					
		very low					1
		low					2
		medium					3
		high					4
		very high					5
11.	**(*)** **QN** **MS/VG**			**(a), (b)**	**57-59**		
		Leaf: size					
		small					3
		medium					5
		large					7
12.	**QN** **VG**			**(a)**	**59-61**		
		Stem: hairiness at the top (last 5 cm)					
		absent or very weak					1
		weak					2
		medium					3
		strong					4
		very strong					5
13.	**(*)** **QN** **MG/MS**		**(+)** **(a)**	**61**			
		Time of beginning of flowering					
		very early					1
		early					3
		medium					5
		late					7
		very late					9

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
14.	**QN VG**	**(+) (a)**	**63-65**			
	NEW! Flower: attitude of ray florets in relation to the head					
	parallel					1
	parallel to right angle					2
	right angle					3
15.	**QN VG**	**(a)**	**63-65**			
	Ray florets: density					
	sparse					3
	medium					5
	dense					7
16.	**QN VG**	**(+) (a)**	**63-65**			
	Ray floret: shape					
	fusiform					1
	narrow ovate					2
	broad ovate					3
	rounded					4
17.	**QL VG**	**(a)**	**63-65**			
	Ray floret: disposition					
	flat					1
	longitudinal recurved					2
	undulated					3
	strongly recurved to back of head					4
18.	**QN VG**	**(a)**	**63-65**			
	Ray floret: length					
	short					3
	medium					5
	long					7

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
19.	**(*)**	**QL**	**VG**		**(a)**	**63-65**			
		Ray floret: color							
		yellowish white							1
		light yellow							2
		medium yellow							3
		orange yellow							4
		orange							5
		purple							6
		reddish brown							7
		multicolored							8
20.		**QL**	**VG**		**(a)**	**63-65**			
		Disk flower: color							
		yellow							1
		orange							2
		purple							3
21.		**QN**	**VG**	**(+)**	**(a)**	**63-65**			
		Disk flower: anthocyanin coloration of stigma							
		absent or very weak							1
		weak							2
		medium							3
		strong							4
		very strong							5
22.	**(*)**	**QL**	**VG**		**(a)**	**63-65**			
		Disk flower: production of pollen							
		absent							1
		present							9
23.		**QN**	**VG**	**(+)**	**(a)**	**63-65**			
		Bract: shape							
		clearly elongated							1
		neither clearly elongated nor clearly rounded							2
		clearly rounded							3

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
24.		**QN VG**	**(+) (a)**	**63-65**			
		Bract: length of tip					
		short					3
		medium					5
		long					7
		very long					9
25.		**QL VG**	**(a)**	**69-73**			
		Bract: attitude in relation to head					
		not embracing or very slightly embracing					1
		slightly embracing					2
		strongly embracing					3
26.	**(*)**	**QN MS**	**(a)**	**69-73**			
		Plant: natural height					
		dwarf					1
		dwarf to extremely short					2
		extremely short					3
		extremely short to very short					4
		very short					5
		very short to short					6
		short					7
		short to medium					8
		medium					9
		medium to tall					10
		tall					11
		tall to very tall					12
		very tall					13
		very tall to extremely tall					14
		extremely tall					15
		extremely tall to giant					16
		giant					17

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
27.	**(*)**	**QL**	**VG**		**(a)**	**69-89**			
		Plant: branching							
		absent							1
		present							9
28.	**(*)**	**QL**	**VG**	**(+)**	**(a)**	**69-89**			
		Plant: type of branching							
		only basal							1
		predominantly basal							2
		overall							3
		predominantly apical							4
		only apical							5
29.		**QL**	**VG**			**69-89**			
		Plant: natural position of highest lateral head to the central head							
		below							1
		same level							2
		above							3
30.	**(*)**	**QL**	**VG**	**(+)**	**(a)**	**80-89**			
		(OLD 32 Part 1) Stem: attitude							
		straight							1
		slightly curved							2
		strongly curved							3
		over turned							4
31.	**(*)**	**QL**	**VG**	**(+)**	**(a)**	**80-89**			
		(OLD 32 Part 2) Head: attitude							
		horizontal							1
		inclined							2
		vertical							3
		half-turned down							4
		turned down							5
		over turned							6

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
32.	**(*)**	**QN MS/VG**	**(a)**	**80-89**			
		Head: size					
		small					3
		medium					5
		large					7
33.	**(*)**	**QL VG**	**(+) (a)**	**85-87**			
		Head: shape of grain side					
		strongly concave					1
		weakly concave					2
		flat					3
		weakly convex					4
		strongly convex					5
		deformed					6
34.		**QN VG**		**99**			
		Seed: size					
		small					3
		medium					5
		large					7
		very large					9
35.	**(*)**	**PQ VG**	**(+)**	**99**			
		Seed: shape					
		elongated					1
		narrow ovoid					2
		broad ovoid					3
		rounded					4
36.		**PQ VG**		**99**			
		Seed: thickness relative to width					
		very thin					1
		thin					2
		medium					3
		thick					4
		very thick					5

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
37.	**(*)** **QL** **VG**		**(+)**	**99**			
		Seed: main color					
		white					1
		whitish grey					2
		grey					3
		light brown					4
		medium brown					5
		dark brown					6
		black					7
		purple					8
38.	**(*)** **QN** **VG**		**(+)**	**99**			
		Seed: stripes on margin					
		none or very weakly expressed					1
		weakly expressed					2
		strongly expressed					3
39.	**(*)** **QN** **VG**		**(+)**	**99**			
		Seed: stripes between margins					
		none or very weakly expressed					1
		weakly expressed					2
		strongly expressed					3
40.	**(*)** **QL** **VG**			**99**			
		Seed: color of stripes					
		white					1
		grey					2
		brown					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) All observations should be made on the main stem.

(b) All observations on the leaf should be made on fully developed leaves at the 2/3 height of the plant, after bud stage but before the flowering stage. The bud should reach a size of about 5 cm.

8.2 Explanations for individual characteristics

Ad. 4: Leaf: serration

Drawing will be presented by HU.

Ad. 5: Leaf: shape of cross section

Drawing will be presented by HU.

Ad. 6: Leaf: shape of distal part

Drawing will be presented by HU.

Ad. 7: Leaf: auricules

Drawing will be presented by HU.

Ad. 8: Leaf: wings

Drawing will be presented by HU.

Ad. 9: Leaf: angle of lowest lateral veins

Drawing will be presented by HU.

Ad. 10: Leaf: height of the tip of the blade compared to insertion of petiole

Drawing will be presented by HU.

Ad. 13: Time of beginning of flowering

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

Ad. 14: NEW! Flower: attitude of ray florets in relation to the head

1
parallel

3
right angle

Ad. 16: Ray floret: shape

Drawing will be presented by HU.

Ad. 21: Disk flower: anthocyanin coloration of stigma

The anthocyanin coloration should be recorded on the stigma from the central third of the head just after the pollen appears at the top of the anthers.
Drawing will be presented by HU.

Ad. 23: Bract: shape

Drawing will be presented by HU.

Ad. 24: Bract: length of tip

Drawing will be presented by HU.

Ad. 28: Plant: type of branching

Drawing will be presented by HU.

Ad. 30: (OLD 32 Part 1) Stem: attitude

Drawing will be presented by HU.

Ad. 31: (OLD 32 Part 2) Head: attitude

Drawing will be presented by HU.

Ad. 33: Head: shape of grain side

Drawing will be presented by HU.

Ad. 35: Seed: shape

Drawing will be presented by HU.

Ad. 37: Seed: main color

The main color of the seed is the color with the largest area. In case of doubt which is the largest area, the darkest color is the main color.

Ad. 38: Seed: stripes on margin

Drawing will be presented by HU.

Ad. 39: Seed: stripes be-tween margins

Drawing will be presented by HU.

8.3 **Sunflower** Weber and Bleiholder, 1990; Lancashire et al., 1991

Phenological growth stages and BBCH-identification keys of sunflower
(Helianthus annuus L.)

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

10. Technical Questionnaire

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

	Application date: (not to be filled in by the applicant)

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

1. Subject of the Technical Questionnaire

1.1 Botanical name	*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 varieties)

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

4.1.3 Mutation [ ]
(please state parent variety)

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 Other [ ]

(Please provide details)

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**		
	weak		3 [ ]
	medium		5 [ ]
	strong		7 [ ]
5.2 (3)	**Leaf: blistering**		
	absent or very weak		1 [ ]
	weak		3 [ ]
	medium		5 [ ]
	strong		7 [ ]
	very strong		9 [ ]
5.3 (13)	**Time of beginning of flowering**		
	very early		1 [ ]
	early		3 [ ]
	medium		5 [ ]
	late		7 [ ]
	very late		9 [ ]
5.4 (19)	**Ray floret: color**		
	yellowish white		1 [ ]
	light yellow		2 [ ]
	medium yellow		3 [ ]
	orange yellow		4 [ ]
	orange		5 [ ]
	purple		6 [ ]
	reddish brown		7 [ ]
	multicolored		8 [ ]
5.5 (22)	**Disk flower: pro-duction of pollen**		
	absent		1 [ ]
	present		9 [ ]

	Characteristics	Example Varieties	Note
5.6 (26)	**Plant: natural height**		
	dwarf		1 [ ]
	dwarf to extremely short		2 [ ]
	extremely short		3 [ ]
	extremely short ot very short		4 [ ]
	very short		5 [ ]
	very short to short		6 [ ]
	short		7 [ ]
	short to medium		8 [ ]
	medium		9 [ ]
	medium to tall		10 [ ]
	tall		11 [ ]
	tall to very tall		12 [ ]
	very tall		13 [ ]
	very tall to extremely tall		14 [ ]
	extremely tall		15 [ ]
	extremely tall to giant		16 [ ]
	giant		17 [ ]
5.7 (27)	**Plant: branching**		
	absent		1 [ ]
	present		9 [ ]
5.8 (37)	**Seed: main color**		
	white		1 [ ]
	whitish grey		2 [ ]
	grey		3 [ ]
	light brown		4 [ ]
	medium brown		5 [ ]
	dark brown		6 [ ]
	black		7 [ ]
	purple		8 [ ]
5.9 (38)	**Seed: stripes on margin**		
	none or very weakly expressed		1 [ ]
	weakly expressed		2 [ ]
	strongly expressed		3 [ ]

	Characteristics	Example Varieties	Note
5.10 (39)	**Seed: stripes be-tween margins**		
	none or very weakly expressed		1 [ ]
	weakly expressed		2 [ ]
	strongly expressed		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			

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

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

[Annex follows]

ANNEX I

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
41	**Allele expression at locus Me1**	Genotype 2/2		1
		Genotype 4/4		2
		Genotype 2/4		3
42	**Allele expression at locus Pgd1**	Genotype 2/2		1
		Genotype 4/4		2
		Genotype 2/4		3
43	**Allele expression at locus Pgi2**	Genotype 2/2		1
		Genotype 4/4		2
		Genotype 2/4		3
44	**Allele expression at locus Shdh1**	Genotype 2/2		1
		Genotype 4/4		2
		Genotype 2/4		3
45	**Allele expression at locus Pgm4**	Genotype 2/2		1
		Genotype 4/4		2
		Genotype 2/4		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$

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 4

6.1.2 Schematization of the zymogrammes

Picture

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 4

6.2.2 Schematization of the zymogrammes

Picture

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 4

6.3.2 Schematization of the zymogrammes

Picture

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 4

6.4.2 Schematization of the zymogrammes

Picture

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 4

6.5.2 Schematization of the zymogrammes

Picture

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.

ANNEX II

1. Introduction

The following Annex contains useful, recognised additional characteristics which might be used for establishing distinctness in cases no difference can be found on the bases of the “normal” characteristics.
UPOV decided to place these characteristics in an Annex to the Test Guidelines, thereby creating a special category of characteristic, because special tests need to be established for observing these characteristics. These characteristics should therefore only be used on the request of the applicant in case the candidate variety is not distinct according to the “normal” characteristics. UPOV reconfirms that these characteristics are considered useful but that they might be unnecessary to observe 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.

2. Table of Characteristics

		Characteristics	Example varieties	Note
46.1	**VS (a) (b)**	**Resistance to downy mildew (Plasmopara halstedii) strain 1 (100)**		
QL		absent		1
		present		9
46.2	**VS (a) (b)**	**Resistance to downy mildew (Plasmopara halstedii) strain 3 (700)**		
QL		absent		1
		present		9
46.3	**VS (a) (b)**	**Resistance to downy mildew (Plasmopara halstedii) strain 4 (730)**		
QL		absent		1
		present		9
46.4	**VS (a) (b)**	**Resistance to downy mildew (Plasmopara halstedii) strain 8 (710)**		
QL		absent		1
		present		9
46.5	**VS (a) (b)**	**Resistance to downy mildew (Plasmopara halstedii) strain 9 (330)**		
QL		absent		1
		present		9
47.1 (+)	**VS (a)**	**Resistance to broomrape (Orobanche spp.) race E**		
QL		absent		1
		present		9
48 (+)	**VS**	**Plant: herbicide tolerance: Imidazolinone**		
QL		sensitive		1
		tolerant		9
49 (+)	**VS**	**Plant: herbicide tolerance: Sulfonilurea**		
QL		sensitive		1
		tolerant		9

Explanations to the Table of Characteristics:

(a), (b): Explanations to several characteristics.
(+): Explanations to single characteristics.

3. Explanations to the Table of Characteristics

3.1 Explanations to several characteristics

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

(a) Resistance to diseases:

If such characteristics are used for the assessment of distinctness, uniformity and stability, infection has to be made under controlled circumstances and with determined pathotypes.

(b) Resistance to downy mildew (Plasmopara halstedii) strains:

Examination of strains and marking of results shall be made separately.

The green house provoking test including the maintenance of Plasmopara halstedii strains and the completion of artificial infections shall be carried out according to the methodology elaborated and efficiently drawn up in the Department of Plant Protection of the Szent István University (Virányi, 2005):

I. Providing of identified Plasmopara halstedii strains (virulence phenotypes)
Pathotype ranking of P. halstedii strains used for the resistance test has been determined for 9 internationally accepted sunflower differentiating lines by means of infection since 1998 (Tourville et. al., 2000). The pathotype against which all of them are susceptible or resistant is known and as regards their majority, P1 gene(s) providing the resistance is/are also of common knowledge. USDA, INRA as well as IFVC (Novi Sad) are the original sources of these lines.
Fungus strains determined for pathotype (virulence phenotype) and repeatedly controlled from time to time are stored in ultra deep freezer (-70^{o}C) and in order to get fresh fungus spore, they are infected on susceptible sunflower (not containing P1 gene) prior to the resistance test.

II. Preparation of sunflower seeds for resistance test
Seeds of sunflower varieties (lines) to be included in the examination shall be steeped for 5 minutes in commercial hypo solution of 15% dilution (final concentration: approx. 1%) to remove bacteria and fungus formulae from the achene shell surface. Flush with flowing water will be made subsequently, then seeds wrapped in thoroughly damped rough filter-paper are germinated for 2-3 days (rootlets of 5-10 mm are the most suitable for the infection).

III. Production of inoculum
Zoosporangia from cotyledons showing fresh sporulation shall be washed in twice distilled water and filtered through antiseptic gauze to prepare clear zoosporangia suspension. Bürker chamber is used to determine the spore count in the suspension and by means of the required dilution zoosporangia suspension of 30-50000 zoosporangia/ml density shall be prepared. When counting, totally light and swollen (living and germinative) zoosporangia are only considered.

IV. Inoculation

Individuals in adequate number and possibly of the same development shall be selected from the germinated seeds of sunflower variety /line. Zoosporangia suspension prepared from the given fungus strain (pathotype) is to be poured on the individuals placed in Petri dishes. Attention shall be paid that all the germs in the Petri dish swim freely and none of them ‘’sink down”. Inoculation shall be made in 16-17^{o}C thermostat for 5-6 hours. This is the so-called whole seedling inoculation, shortly WSI (Cohen és Sackston, 1973).

V. Greenhouse plant growing

Inoculated sunflower germs are planted in propagating box, or pot previously filled with clean, pathogen free vegetable soil. Sunflowers are grown at the temperature of 17-26^{o}C (night/day), in a photoperiod of 14-16 hours with lighting of about 12-16000 lux.

VI. Evaluation and interpretation

First evaluation based on the sporulation of pathogen is generally carried out 8-10 days after planting. For this purpose boxes (plots) should be placed in vaporous area and temperature possibly kept at 18-l9^{o}C. Sunflower varieties/lines are to be assessed next morning on the basis of zoosporangia cover appeared on seminal leaves and maybe on the first foliage-leaves of the plants. However the determination of sporulation is not sufficient to judge the susceptibility or resistance (seminal leaves of some genotypes are susceptible, but other parts of the plant are resistant) (Vear, 1978; Virányi, 1978), therefore after an additional growing of 7-10 days plants shall be assessed once again. When the second assessment is carried out, plants with foliage-leaves showing typical chlorotic contours, the sure symptoms of systematic infection are counted. In the case of strong infection, or excessively susceptible variety one part of diseased plants, similarly to plantlet lodging, can die out by the time of the second assessment. After all the reaction of the tested sunflower variety/line against the given pathotype of the pathogen (susceptibility or resistance) is indicated by the percental value computed from the total number of plants showing sporulation and/or leaf chlorosis and those that were died out. Optimally about one-hundred-per-cent disease indicates susceptibility, while symptom-free status is the measure of resistance. In the case of intermediate values the lack of balance of plant material may be considered.

3.2 Explanations to single characteristics

55 Resistance to broomrape (Orobanche spp.) race E:

Green house provoking test including the maintenance of broomrape race E and the performance of artificial infection shall be carried out on the basis of the following method:

Required seed quantity (number of achene): 30 pcs/genotype.

Inoculum: domestic sunflower broomrape (Orobanche cumana) population containing race E with at least 250 mg broomrape seed / 1kg sand quantity.

Per genotype 20-20 plants are grown in plastic pots of 15 cm in diameter (5 plants/pot, 4 replications) filled to three-quarters with calciferous sand containing the inoculum ($CaCO_3$ content: min. 6%). Sunflower seeds placed on the surface of sand are covered in 3 cm thickness with peaty mould. Plants are grown for 6 weeks in glasshouse conditions with regular watering.

Judgement of contamination: 3-4 days prior to the evaluation (40th day after sowing) watering is stopped and when the sand is dried out, judgement is carried out by visual symptomatic evaluation of roots lifted out of the sand in the following way:

Resistance: present (note 9): broomrape free root system and/or necrotic spots on the root system,
Resistance: absent (note 1): presence of broomrape on the root system.

9 – present

1 - absent

57 Plant: herbicide tolerance: Imidazolinone:

Field test. Duration of tests is normally one growing cycle. Tests should be performed normally in one location.
Test should include at least 40 plants divided into two or more replicates.
Effective agent of the product included in the trial: Imazamox.
Method of treatment: post-merged full surface spraying.
Time of treatment: development in 4 true-leaves of sunflower (BBCH 14).
Time of evaluation: 7, 14 and 21 days after treatment.

Evaluation of the phytotoxic effect:

When judging the phytotoxic effect, the cultivated plant is examined in the plot as individual and population. Strength of visible symptom is determined for the cultivated plant and it is expressed in the percentage compared to the untreated (hacked as circumstances require) control. Strength of visible phytotoxic symptoms (discolorations, deformations, growth inhibition, etc.) developed on the cultivated plants shall be deermined in terms of percentage considering the following two factors:

- percentage of cultivated plants showing the symptoms,
- extent of damage of individual plants.

The determination of the extent of damage is made by means of one whole percentage number and the two factors are jointly regarded. On the basis of agreement the percentage values of the phytotoxic effect divide into 9 value ranges described with text. Description of the categories with text is carried out on the basis of classification as follows (percentage value numbers of the category are the highest ones belonging to the given category).

Fitox%	Description
0	symptom-free
1	very mild symptom
2	mild symptom
5	definite symptom
10	damaged
25	strong damage
50	heavy damage
75	very heavy damage
100	died out

Variety is tolerant (note 9): Fitox% <= 5%,
Variety is sensitive (note 1): Fitox% > 5%.

1
sensitive

9
tolerant

58 Plant: herbicide tolerance: Sulfonilurea:

Field test. The duration of tests is normally one growing cycle. Tests should be performed normally in one location.
Test should include at least 40 plants divided into two or more replicates.
Effective agent of the product included in the trial: Tribenuron-metyl.
Method of treatment: post-merged full surface spraying.
Time of treatment: development in 4 true-leaves of sunflower (BBCH 14).
Time of evaluation: 7, 14 and 21 days after treatment.

Evaluation of the phytotoxic effect:

When judging the phytotoxic effect, the cultivated plant is examined in the plot as individual and population. Strength of visible symptom is determined for the cultivated plant and it is expressed in the percentage compared to the untreated (hacked as circumstances require) control. Strength of visible phytotoxic symptoms (discolorations, deformations, growth inhibition, etc.) developed on the cultivated plants shall be deermined in terms of percentage considering the following two factors:

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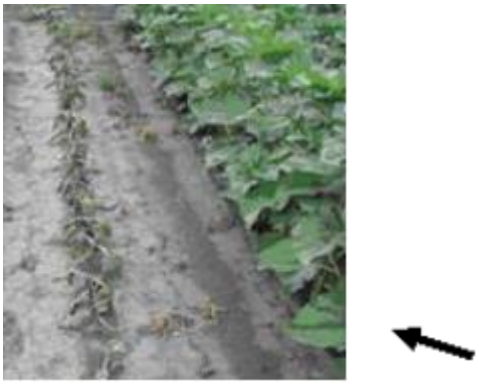
Variety is sensitive (note 1): Fitox% > 5%.



1 - sensitive 9 - tolerant

[End of Annex and of document]