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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 fiftieth session, to be held in Arusha, United Republic of Tanzania,
from 2021-06-21 to 2021-06-25

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.]

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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.

4.2.7 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 11)
(d) Ray floret: color (characteristic 17)
(e) Disk flower: production of pollen (characteristic 20)
(f) Only inbred lines: Plant: natural height (characteristic 25)
(g) Only hybrids and open-pollinated varieties: Plant: natural height (characteristic 26)
(h) Plant: branching (characteristic 27)
(i) Seed: color (characteristic 37)
(j) Seed: stripes on margin (characteristic 38)
(k) Seed: stripes between 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 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 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 8.2

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

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1.		**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					
		light				F5DN3MA, T0243HG	1
		medium				H11050R	3
		dark				13013	5
3.	**(*)**	**QN VG**		**(a), (b)**	**51-55**		
		Leaf: blistering					
		absent or very weak				F5DN3MA	1
		weak				F7AX2JA, IR79DMR	3
		medium				HA89, IB1088DMR	5
		strong				TRC2342	7
		very strong					9
4.	**(*)**	**QN VG**	**(+)**	**(a), (b)**	**51-55**		
		Leaf: serration					
		isolated or very fine				99D40R	1
		fine				IR79DMR	3
		medium				HA89, TRC2342	5
		coarse				PB1458DMR	7
		very coarse					9
5.		**QN VG**	**(+)**	**(a), (b)**	**53-55**		
		Leaf: shape in cross section					
		concave				RT9513	1
		flat				PH5002R	3
		convex					5

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
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
		short acuminate					6
		broad triangular to rounded				SF9074MA	7
		medium acuminate					8
		rounded					9
7.	**(*)**	**QN VG**	**(+)**	**(a), (b)**	**53-55**		
		Leaf: auricules					
		none or very small				37025	1
		small				T0954LM	3
		medium					5
		large				F6AH6MO, HA89	7
		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

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
10	**(*)**	**QN MS/VG**	**(a), (b)**	**55-57**			
		Leaf: size					
		small				PH5002R	3
		medium				LC1093, OB724	5
		large				IA1169DMR	7
11	**(*)**	**QN MG/MS**	**(+) (a)**	**61**			
		Time of beginning of flowering					
		very early				PHA283	1
		early				T0860LM	3
		medium				H11050R, RHA274	5
		late				RT7710	7
		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: disposition					
		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
		medium				99D40R, HA89	3
		very dense				OB724	5
15		**QN MS/VG**	**(a)**	**63-65**			
		Ray floret: length					
		very short				BT0835	1
		medium				SF9074MA	3
		very long				T0954LM	5

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
16		**QN**	**MS/VG**	**(+)**	**(a)**	**63-65**			
		Ray floret: width in relation to length							
		very narrow						T0954LM	1
		narrow						HA850, OB724	2
		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		**PQ**	**VG**		**(a)**	**63-65**			
		Disk flower: color							
		yellow						STR226, TRC2342	1
		orange						F7AW1MOA, HA89	2
		purple							3
19		**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
20	**(*)**	**QL**	**VG**		**(a)**	**63-65**			
		Disk flower: production of pollen							
		absent						F7AW1MOA, HA89	1
		present						IR79DMR, RHA274	9

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
21		**QN**	**VG**	**(+)**	**(a)**	**63-65**			
		Bract: shape							
		clearly elongated						T0954LM	1
		neither clearly elongated nor clearly rounded						IR79DMR	2
		clearly rounded						IB1088DMR	3
22		**QN**	**MS/VG**	**(+)**	**(a)**	**63-65**			
		Bract: length of tip							
		very short						IB1088DMR	1
		medium						HA89, T0954LM	3
		very long						U0881BG	5
23		**QN**	**VG**		**(a)**	**63-65**			
		Bract: intensity of green color of outer side							
		light						T0243HG	1
		medium						T0954LM	2
		dark						RT8711	3
24		**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
25	**(*)**	**QN**	**MS**		**(a)**	**69-73**			
		<u>Only inbred lines:</u> Plant: natural height							
		very short						FR810RM1	1
		short						OB724	3
		medium						U0881BG	5
		tall						R6ST2MI	7
		very tall						31G03	9

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
26	**(*)**	**QN**	**MS**			**69-73**			
		<u>Only hybrids and open-pollinated varieties:</u> Plant: natural height							
		very short						Antonil	1
		short						GK Milia	3
		medium						Sumiko	5
		tall						Marley	7
		very tall						Kisvárdai	9
27	**(*)**	**QL**	**VG**		**(a)**	**69-89**			
		Plant: branching							
		absent						HA89, OB724	1
		present						RHA274, T0954LM	9
28	**(*)**	**PQ**	**VG**	**(+)**	**(a)**	**69-89**			
		<u>Only varieties with Plant: branching: present:</u> Plant: type of branching							
		only basal							1
		predominantly basal							2
		overall						H11050R	3
		predominantly apical						RHA274, T0954LM	4
		only apical						TRC2342	5
29		**QN**	**VG**			**69-89**			
		<u>Only varieties with Plant: branching: present:</u> Plant: position of highest lateral head to central head							
		below						PH5004R	1
		same level						T0954LM	2
		above						99D40R	3
30	**(*)**	**QN**	**VG**		**(a)**	**80-89**			
		Stem: attitude							
		straight						U0881BG	1
		slightly curved							2
		strongly curved						F7EW2MIA	3
		over turned							4

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
31	**(*)**	**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
32	**(*)**	**QN**	**MS/VG**		**(a)**	**80-89**			
		Head: size							
		small						RT0976	3
		medium						BT0835, HA89	5
		large						F5DN3MA	7
33	**(*)**	**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
34		**QN**	**MS/VG**		**(a)**	**99**			
		Seed: size							
		very small						PHA283	1
		small						TRC2342	3
		medium						HA89, OB724	5
		large						FT2603, Kisvárdai	7
		very large							9
35	**(*)**	**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 Exemples Beispielssorten Variedades ejemplo	Note/ Nota
36		**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
37	**(*)**	**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
38	**(*)**	**QN VG**	**(+) (a)**	**99**			
		Seed: stripes on margin					
		none or very weakly expressed				T0954LM	1
		weakly expressed				OB724	2
		strongly expressed				HA89, U0881BG	3
39	**(*)**	**QN VG**	**(+) (a)**	**99**			
		Seed: stripes between margins					
		none or very weakly expressed				T0954LM	1
		weakly expressed				LGR27	2
		strongly expressed				HA89, U0881BG	3

		English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
40	**(*)**	**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) 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.

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
concave	flat	convex

Ad. 6: Leaf: shape of distal part

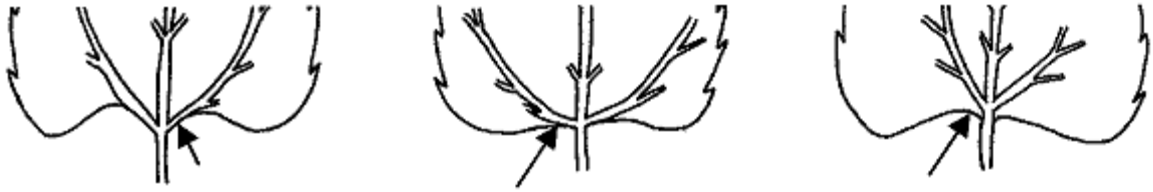
1	3	5	8	9
lanceolate	narrow triangular	broad triangular	medium acuminate	rounded

Ad. 7: Leaf: auricules

1	3	5	7	9
none or very small	small	medium	large	very large

Ad. 8: Leaf: wings

(parenchym at base of lateral veins)



1	2	3
none or very weakly expressed	weakly expressed	strongly expressed

Ad. 9: Leaf: angle of lowest lateral veins

1	2	3
acute	right angle or nearly right angle	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: disposition

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
narrow

3
broad

4
very broad

Ad. 17: Ray floret: color

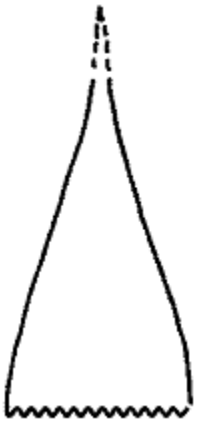
If more than one color, only the color covering the biggest surface is considered.

Ad. 19: Disk flower: anthocyanin coloration of stigma

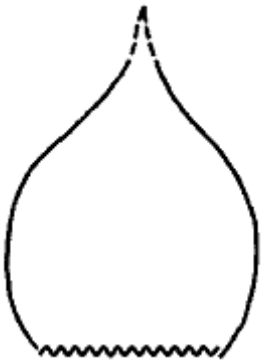
The anthocyanin coloration should be recorded on the stigma just after the pollen appears at the top of the anthers.

Ad. 21: Bract: shape

1
clearly elongated

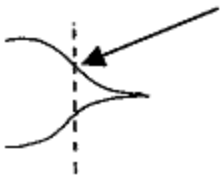


2
neither clearly elongated nor clearly rounded



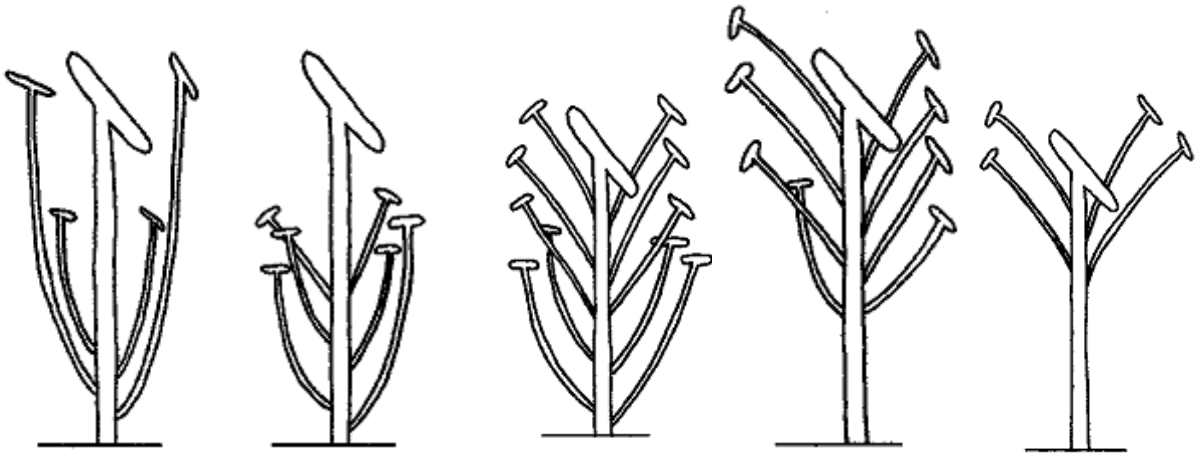
3
clearly rounded

Ad. 22: Bract: length of tip



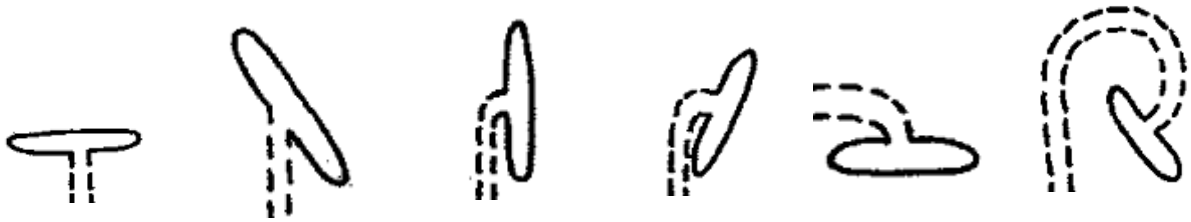
Tip begins where the direction of curving changes

Ad. 28: Only varieties with Plant: branching: present: Plant: type of branching



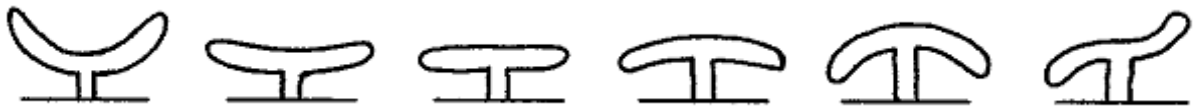
1	2	3	4	5
only basal	predominantly basal	overall	predominantly apical	only apical

Ad. 31: Head: attitude



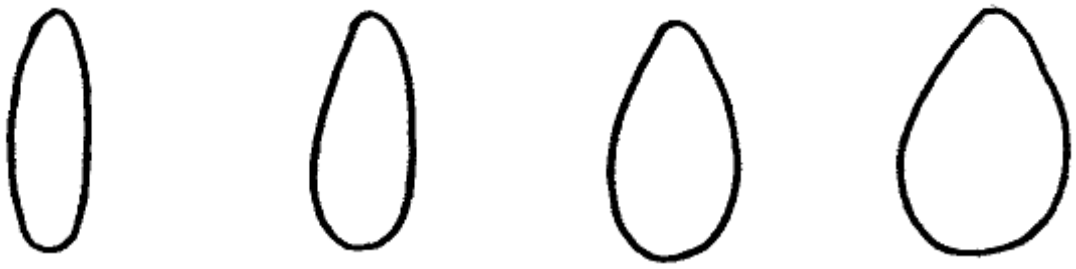
1	2	3	4	5	6
horizontal	inclined	vertical	half-turned down	turned down	over turned

Ad. 33: Head: shape of grain side



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

Ad. 35: Seed: shape



1	2	3	4
elongated	narrow ovoid	broad ovoid	rounded

Ad. 37: Seed: 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

Ad. 39: 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: (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	[ ]
(b)	Hybrid	[ ]
(i)	Single 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) maintenance system of male sterile lines.

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**		
	light	F5DN3MA, T0243HG	1 [ ]
	medium	H11050R	3 [ ]
	dark	13013	5 [ ]
5.2 (3)	**Leaf: blistering**		
	absent or very weak	F5DN3MA	1 [ ]
	weak	F7AX2JA, IR79DMR	3 [ ]
	medium	HA89, IB1088DMR	5 [ ]
	strong	TRC2342	7 [ ]
	very strong		9 [ ]
5.3 (11)	**Time of beginning of flowering**		
	very early	PHA283	1 [ ]
	early	T0860LM	3 [ ]
	medium	H11050R, RHA274	5 [ ]
	late	RT7710	7 [ ]
	very late	Kisvárdai, LGR27	9 [ ]
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 (20)	**Disk flower: production of pollen**		
	absent	F7AW1MOA, HA89	1 [ ]
	present	IR79DMR, RHA274	9 [ ]

	Characteristics	Example Varieties	Note
5.6 (25)	**<u>Only inbred lines:</u> Plant: natural height**		
	very short	FR810RM1	1 [ ]
	short	OB724	3 [ ]
	medium	U0881BG	5 [ ]
	tall	R6ST2MI	7 [ ]
	very tall	31G03	9 [ ]
5.7 (26)	**<u>Only hybrids and open-pollinated varieties:</u> Plant: natural height**		
	very short	Antonil	1 [ ]
	short	GK Milia	3 [ ]
	medium	Sumiko	5 [ ]
	tall	Marley	7 [ ]
	very tall	Kisvárdai	9 [ ]
5.8 (27)	**Plant: branching**		
	absent	HA89, OB724	1 [ ]
	present	RHA274, T0954LM	9 [ ]
5.9 (37)	**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 (38)	**Seed: stripes on margin**		
	none or very weakly expressed	T0954LM	1 [ ]
	weakly expressed	OB724	2 [ ]
	strongly expressed	HA89, U0881BG	3 [ ]
5.11 (39)	**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) ornamental
(f) 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

[Annex follows]

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

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 $MgCl_2$ 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 Na_2 salt
60 mg 6-Phosphogluconic acid Na_3 salt
10 mg NADP
1 ml MTT solution (4.3.1.3.)
1.5 ml PMS solution (4.3.1.5.)
1 ml $MgCl_2$ 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 $1H_2O$, Na_2 salt
150 mg EDTA, Na_2
10 mg NADP
1.5 ml MTT solution (4.3.1.3)
1.ml PMS solution (4.3.1.5)
4 ml $MgCl_2$ 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.

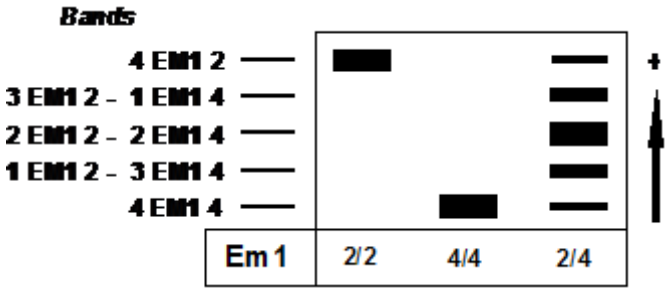
6. Recognition of the alleles encoding isoenzymes

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

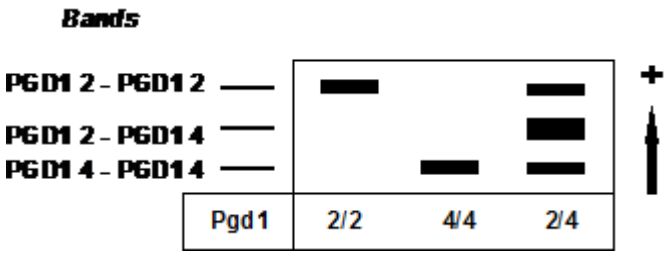


6.2 Recognition of the alleles encoding PGD

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



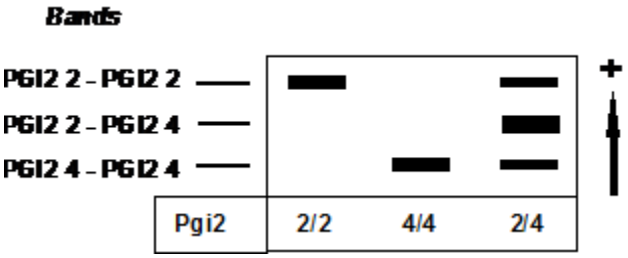
Two migration zones can be observed; only the slowest migrating bands are polymorphic.

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



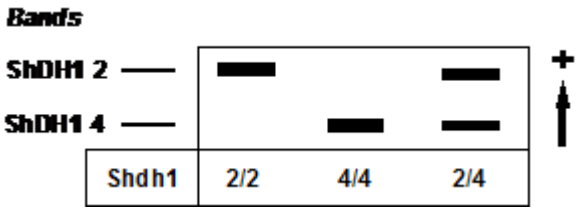
There are two migration zones; only the slowest migrating bands are scored.

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

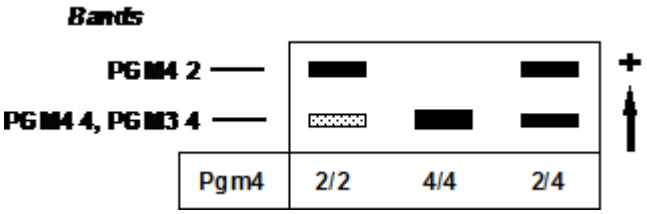


6.5 Recognition of the alleles encoding PGM

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



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.

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