CPVO · OCVV
Community Plant Variety Office
Office Communautaire des Variétés Végétales

PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

***Helianthus annuus* L.**

SUNFLOWER

UPOV Code: HLNTS_ANN

Adopted on 28/01/2026

Entry into force on 15/01/2026

CPVO-TP/081/2 based on the version 5 of the CPVO-TP/template

TABLE OF CONTENTS

CPVO-TP/185/2

1. SUBJECT OF THE PROTOCOL AND REPORTING 3
1.1 Scope of the technical protocol 3
1.2 Entry into Force 3
1.3 Reporting between Examination Office and CPVO and Liaison with Applicant 3
2. MATERIAL REQUIRED 4
2.1 Plant material requirements 4
2.2 Informing the applicant of plant material requirements 4
2.3 Informing about problems on the submission of material 4
3. METHOD OF EXAMINATION 4
3.1 Number of growing cycles 4
3.2 Testing Place 4
3.3 Conditions for Conducting the Examination 4
3.4 Test design 4
3.5 Special tests for additional characteristics 5
3.6 Constitution and maintenance of a variety collection 5
4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY 5
4.1 Distinctness 5
4.2 Uniformity 7
4.3 Stability 7
5. GROUPING OF VARIETIES AND ORGANISATION OF THE GROWING TRIAL 7
6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS 8
6.1 Characteristics to be used 8
6.2. States of expression and corresponding notes 8
6.3 Example Varieties 8
6.4 Legend 9
7. TABLE OF CHARACTERISTICS 10
8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS 21
8.1 Explanations covering several characteristics 21
8.2 Explanations for individual characteristics 21
8.3 Explanations on growth stages 27
ELECTROPHORESIS 28
9. LITERATURE 36
10. TECHNICAL QUESTIONNAIRE 37

1. SUBJECT OF THE PROTOCOL AND REPORTING

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of *Helianthus annuus* L. (**excluding ornamental varieties**).

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on documents agreed by the International Union for the Protection of New Varieties of Plants (UPOV), such as the General Introduction to DUS (UPOV Document TG/1/3 http://www.upov.int/export/sites/upov/resource/en/tg_1_3.pdf), its associated TGP documents (http://www.upov.int/tgp/en/) and the relevant UPOV Test Guideline TG/81/7 dated 31/08/2023 (https://www.upov.int/edocs/tgdocs/en/tg081.pdf) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **15.01.2026**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first growing cycle.

In cases where the Office requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process to be carried out at the moment of this request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

1.3 Reporting between Examination Office and CPVO and Liaison with Applicant

1.3.1 Reporting between Examination Office and CPVO

The Examination Office shall deliver to the CPVO a preliminary report ("the preliminary report") no later than four weeks after the date of the request for technical examination by the CPVO and in any case preferably before the submission period of the plant material.

The Examination Office shall also deliver to the CPVO a report relating to each growing period ("the interim report") and, when the Examination Office considers the results of the technical examination to be adequate to evaluate the variety or the CPVO so requests, a report relating to the examination ("the final report").

The final report shall state the opinion of the Examination Office on the distinctness, uniformity and stability of the variety. Where it considers those criteria to be satisfied, or where the CPVO so requests, a description of the variety shall be added to the report.

In case the variety is an agricultural hybrid, the variety descriptions of the parental components shall be added as well. The variety descriptions of hybrid varieties shall not have any reference to the hybrid formula.

If a report is negative the Examination Office shall set out the detailed reasons for its findings.

The interim and the final reports shall be delivered to the CPVO as soon as possible and no later than on the deadlines as laid down in the designation agreement.

1.3.2 Informing on problems in the DUS test

In cases where the Examination Office identifies issues during the course of the technical examination that may lead to a negative report, the Examination Office shall inform the CPVO and in urgent cases the applicant/holder as soon as such issues become obvious.

1.3.3 Sample keeping in case of problems

As far as feasible the Examination Office shall keep a representative sample of any relevant testing material of the candidate variety and reference variety(ies) if the technical examination has resulted in a negative report. As soon as possible, the CPVO shall inform the Examination Office when the material can be destroyed.

2. MATERIAL REQUIRED

2.1 Plant material requirements

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on https://public.plantvarieties.eu/publication in the special issue S2/S3 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

2.2 Informing the applicant of plant material requirements

The CPVO informs the applicant that:

- he is responsible for ensuring compliance with any customs and plant health requirements;
- the plant material supplied should be visibly healthy, not lacking in vigour, nor affected by any important pest or disease;
- 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.

2.3 Informing about problems on the submission of material

The Examination Office shall report to the CPVO immediately in cases where the test material of the candidate variety has not arrived in time or in cases where the material submitted does not fulfil the conditions laid down in the request for submission of plant material issued by the CPVO.

In cases where the examination office encounters difficulties to obtain plant material of reference varieties, the CPVO should be informed in writing.

3. METHOD OF EXAMINATION

3.1 Number of growing cycles

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

The testing of a variety may be concluded when the entrusted examination office 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" http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf.

3.3 Conditions for Conducting the Examination

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.

The optimum stage of development for the assessment of each characteristic is indicated by a number in the third column of the Table of Characteristics. The stages of development denoted by each number are described in Chapter 8.3.

3.4 Test design

Each test should be designed to result in a total of at least 40 plants, which should be divided between at least 2 replicates.

In case of hybrids, the parental components (including the maintainer line) have to be included in the test and should be tested and assessed as any other variety.

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 Special tests for additional characteristics

In accordance with Article 23 of Implementing Rules N° 874/2009 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characteristics listed in the protocol.

3.6 Constitution and maintenance of a variety collection

The process for the constitution and the maintenance of a variety collection can be summarized as follows:

Step 1: Making an inventory of the varieties of common knowledge.

Step 2: Establishing a collection ("variety collection") of varieties of common knowledge which are relevant for the examination of distinctness of candidate varieties.

Step 3: Selecting the varieties from the variety collection which need to be included in the growing trial or other tests for the examination of distinctness of a particular candidate variety.

3.6.1 Forms of variety collection
The variety collection shall comprise variety descriptions and living plant material, thus a living reference collection. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database.

3.6.2 Living Plant Material
The EO shall collect and maintain living plant material of varieties of the species concerned in the variety collection.

3.6.3 Range of the variety collection
The living variety collection shall cover at least those common knowledge varieties that are suitable to grow in the climatic conditions of a respective EO.

The variety collection shall include all varieties used as parental components of all the hybrid varieties included in the variety collection, as well as varieties of common knowledge in their own right.

3.6.4 Making an inventory of varieties of common knowledge for inclusion in the variety collection
The inventory shall include varieties protected under National and Community PBR, varieties registered in the Common Catalogue, the OECD list, the Conservation variety list.

The inventory shall take into account the list of varieties which are the subject of an on-going application for protection or official registration (candidate varieties).

3.6.5 Maintenance and renewal/update of a living variety collection
The EO shall maintain seeds in conditions which will ensure germination and viability, periodical checks, and renewal as required.
Living material in variety collections representing varieties for which a DUS test was carried out at that EO shall be renewed after verification in a side-by-side comparison. In case where no living material is available anymore in the collection, such verification could be done with any other test that has proven to give similar results between the material in the collection and the new material.

4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

The prescribed procedure is to assess distinctness, uniformity and stability in a growing trial.

4.1 Distinctness

4.1.1 General recommendations
It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 9 'Examining Distinctness' (http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

To assess distinctness of hybrids, a pre-screening system on the basis of the parental lines and the formula may be established according to the following recommendations:

(i) description of parental lines according to the Technical Protocols;

(ii) check of the distinctness of the parental lines in comparison with the reference collection, based on the characteristics in the table of characteristics in order to screen the closest inbred lines;

(iii) check of the distinctness of the hybrid formula in comparison with those of the hybrids in common knowledge, taking into account the closest inbred lines;

(iv) assessment of the distinctness at the hybrid level of 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 Technical Protocols are familiar with the recommendations contained in the UPOV-General Introduction to DUS prior to making decisions regarding distinctness.

If distinctness is assessed using the 2 x 1% criterion, the difference between two varieties is clear if the respective characteristics are significantly different in the same direction at the 1% level in at least two out of three years. The tests in each year are based on Student's two-tailed t-test of the differences between variety means with standard errors estimated using the residual mean square from the analysis of the variety x replicate plot means.

If distinctness is assessed by the combined over years distinctness analysis (COYD) the difference between two varieties is clear if the respective characteristics are different at the 1% significance level or less ($p<0.01$) in a test over either two or three years.

If the significance level or statistical methods prescribed are not appropriate the method used should be clearly described.

4.1.4 Number of plants/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 taken from each of 36 plants and any other observations made on all plants in the test, disregarding any off-type plants.

In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 1.

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 third column of 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., colour 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 this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 10 'Examining Uniformity' (http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

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

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 UPOV-General Introduction to DUS.

Where the assessment of a hybrid variety involves the parental components, 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 parental components.

For the assessment of uniformity of inbred varieties and parental components of hybrid 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 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 hybrid varieties, a population standard of 5% and 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.

If uniformity is assessed by the combined over years uniformity method (COYU) the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the 0,1% significance level or less ($p<0.001$) in a test over two or three consecutive cycles.

4.3 Stability

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 11 'Examining Stability' (http://www.upov.int/edocs/tgpdocs/en/tgp_11.pd)

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.

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.

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 ORGANISATION 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 organise 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 colour (characteristic 2)

b) Leaf: blistering (characteristic 3)

c) Time of beginning of flowering (characteristic 11)

d) Ray floret: colour (characteristic 17)

e) Disc floret: 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: colour (characteristic 37)

j) Seed: stripes on margin (characteristic 38)

k) Seed: stripes between margins (characteristic 39)

5.4 If characteristics other than those mentioned in the list of grouping characteristics and/or from the table of characteristics and/or from the Technical Questionnaire – sections 5 and 7. are used for the selection of varieties to be included into the growing trial, the EO shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.

5.5 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the UPOV-General Introduction to DUS and document TGP/9 "Examining Distinctness".

6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

6.1 Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted or by specific legislation on plant health. In the latter case, the CPVO should be informed.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N°874/2009, to insert additional characteristics and their expressions in respect of a variety.

The list of characteristics derived from electrophoresis as in chapter 9 should only be used as a complement to other differences in morphological or physiological characteristics.

6.2. States of expression and corresponding notes

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

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

6.3 Example Varieties

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

6.4 Legend

For column 'CPVO N°':

G	Grouping characteristic	-see Chapter 5
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	Explanations for individual characteristics	-see Chapter 8.2

For column 'UPOV N°':

The numbering of the characteristics is provided as a reference to the UPOV guideline.

(*)	UPOV Asterisked characteristic	- Characteristics that are important for the international harmonization of variety descriptions.

For column 'Stage, method':

MG, MS, VG, VS		-see Chapter 4.1.5
(a)	Explanations covering several Characteristics	-see Chapter 8.1
00-99	Explanations on growth stages	-see Chapter 8.3

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1.	**1.**	**10**	**Seedling: anthocyanin coloration of hypocotyl**		
QN		**VG**	absent or very weak	T0954LM	1
			weak	OB724	2
			medium	TRC3285	3
			strong	F7AW1MOA	4
			very strong	Kisvárdai	5
2.	**2. (*)**	**51-55**	**Leaf: intensity of green colour**		
QN		**VG**	very light	F5DN3MA, T0243HG	1
		(a)	light		2
			medium	H11050R	3
			dark		4
G			very dark	13013	5
3.	**3. (*)**	**51-55**	**Leaf: blistering**		
QN		**VG**	absent or very weak	F5DN3MA	1
		(a)	very weak to weak		2
			weak	F7AX2JA, IR79DMR	3
			weak to medium		4
			medium	HA89, IB1088DMR	5
			medium to strong		6
			strong	TRC2342	7
			strong to very strong		8
G			very strong	RHA361	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
4. (+)	**4. (*)**	**51-55**	**Leaf: serration**		
QN		**VG**	isolated or very fine	99D40R	1
		(a)	very fine to fine		2
			fine	IR79DMR	3
			fine to medium		4
			medium	HA89, TRC2342	5
			medium to coarse		6
			coarse	PB1458DMR	7
			coarse to very coarse		8
			very coarse		9
5. (+)	**5.**	**53-55**	**Leaf: profile in cross section**		
QN		**VG**	strongly concave	RT9513	1
		(a)	weakly concave		2
			flat	PH5002R	3
			weakly convex	IR199DMR	4
			strongly convex		5
6. (+)	**6.**	**53-55**	**Leaf: shape**		
PQ		**VG**	elliptic	FR810RM1	1
		(a)	very narrow triangular	FR81013	2
			narrow triangular	RT0976	3
			medium triangular	RT9513	4
			broad triangular	BT0835	5
			triangular to rounded		6
			rounded	F1902MJS, F9AZ8M2	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
7. (+)	**7. (*)**	**53-55**	**Leaf: lobes**		
QN		**VG**	absent or very small	37025	1
		(a)	very small to small	TRO5098R	2
			small	T0954LM	3
			small to medium		4
			medium	F9AZ8MJKA	5
			medium to large		6
			large	F6AH6MO, HA89	7
			large to very large		8
			very large	RHA299	9
8. (+)	**8.**	**53-55**	**Leaf: parenchyma at base of lateral veins**		
QN		**VG**	none or very weak	T0954LM	1
		(a)	weak	F7AW1MOA	2
			strong	13013	3
9. (+)	**9. (*)**	**53-55**	**Leaf: angle of lowest lateral veins**		
QN		**VG**	acute	T0860LM	1
		(a)	right angle or nearly right angle	F7AW1MOA	2
			obtuse	TFC3767B	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
10.	**10. (*)**	**55-57**	**Leaf: size**		
QN		**MS/VG**	very small		1
		(a)	very small to small		2
			small	PH5002R	3
			small to medium		4
			medium	LC1093, OB724	5
			medium to large		6
			large	IA1169DMR	7
			large to very large		8
			very large		9
11. (+)	**11. (*)**	**61**	**Time of beginning of flowering**		
QN		**MG/MS**	very early	PHA283	1
			very early to early		2
			early	T0860LM	3
			early to medium		4
			medium	H11050R, RHA274	5
			medium to late		6
			late	RT7710	7
			late to very late		8
G			very late	Kisvárdai, LGR27	9
12. (+)	**12.**	**63-65**	**Ray floret: attitude of base in relation to head**		
QN		**VG**	erect	T0833HG	1
			semi-erect		2
			horizontal	T0954LM	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
13. (+)	**13.**	**63-65**	**Ray floret: profile**		
PQ		**VG**	flat	HA89, IR79DMR	1
			rolled	PH5002R	2
			twisted	F5DN3MA	3
			strongly recurved	BT0833	4
14.	**14.**	**63-65**	**Flower: density of ray florets**		
QN		**VG**	very sparse	T0954LM	1
			sparse		2
			medium	99D40R, HA89	3
			dense		4
			very dense	OB724	5
15.	**15.**	**63-65**	**Ray floret: length**		
QN		**MS/VG**	very short	BT0835	1
			short		2
			medium	SF9074MA	3
			long		4
			very long	T0954LM	5
16. (+)	**16.**	**63-65**	**Ray floret: width in relation to length**		
QN		**MS/VG**	very narrow	T0954LM	1
			narrow	HA850, OB724	2
			broad	F71539	3
			very broad	HP4128IOA	4

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
17. (+)	**17. (*)**	**63-65**	**Ray floret: colour**		
PQ		**VG**	yellowish white	RHA381	1
			light yellow	F7AW1MOA	2
			medium yellow	RT7710	3
			orange yellow	U0881BG	4
			orange	OB724, P211R	5
			purple		6
G			reddish brown		7
18.	**19.**	**63-65**	**Disc floret: colour**		
PQ		**VG**	yellow	STR226, TRC2342	1
			orange	F7AW1MOA, HA89	2
			purple		3
19. (+)	**21.**	**63-65**	**Disc floret: anthocyanin coloration of stigma**		
QN		**VG**	absent or very weak	SF9074MA	1
			weak	RT7710	2
			medium	R6ST2MI, TRC2342	3
			strong	F7AW1MOA	4
			very strong	Kisvárdai	5
20.	**22. (*)**	**63-65**	**Disc floret: production of pollen**		
QL		**VG**	absent	F7AW1MOA, HA89	1
G			present	IR79DMR, RHA274	9
21. (+)	**23.**	**63-65**	**Bract: shape**		
PQ		**VG**	narrow acute	T0954LM	1
			broad acute	IR79DMR	2
			rounded	IB1088DMR	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
22. (+)	**24.**	**63-65**	**Bract: length of tip**		
QN		**MS/VG**	very short	IB1088DMR	1
			short		2
			medium	HA89, T0954LM	3
			long		4
			very long	U0881BG	5
23.	**25.**	**63-65**	**Bract: intensity of green colour of outer side**		
QN		**VG**	light	T0243HG	1
			medium	T0954LM	2
			dark	RT8711	3
24.	**26.**	**69-73**	**Bract: attitude in relation to head**		
QN		**VG**	not adpressed or very slightly adpressed	HA89, RT0976	1
			slight adpressed	F7AW1MOA	2
			strongly adpressed	RT9513	3
25.	**27. (*)**	**69-73**	**<u>Only inbred lines</u>: Plant: natural height**		
QN		**MS**	very short	FR810RM1	1
			very short to short		2
			short	OB724	3
			short to medium		4
			medium	U0881BG	5
			medium to tall		6
			tall	R6ST2MI	7
			tall to very tall		8
G			very tall	31G03	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
26.	**28. (*)**	**69-73**	**Only hybrids and open-pollinated varieties: Plant: natural height**		
QN		**MS**	very short	Antonil	1
			very short to short		2
			short	GK Milia	3
			short to medium		4
			medium	Sumiko	5
			medium to tall		6
			tall	Marley	7
			tall to very tall		8
G			very tall	Kisvárdai	9
27.	**29. (*)**	**69-89**	**Plant: branching**		
QL		**VG**	absent	HA89, OB724	1
G			present	RHA274, T0954LM	9
28. (+)	**30. (*)**	**69-89**	**Only varieties with Plant: branching: present: Plant: position of branching**		
PQ		**VG**	only basal		1
			predominantly basal	RHA293	2
			throughout	H11050R	3
			predominantly apical	RHA274, T0954LM	4
			only apical	TRC2342	5
29.	**31.**	**69-89**	**Only varieties with Plant: branching: present: Plant: position of highest lateral head to central head**		
QN		**VG**	below	PH5004R	1
			same level	T0954LM	2
			above	99D40R	3

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
30. (+)	**32. (*)**	**80-89**	**Stem: attitude**		
QN		**VG**	straight	U0881BG	1
			slightly curved	R14WZ3MJI	2
			strongly curved	F7EW2MIA	3
31. (+)	**33. (*)**	**80-89**	**Head: attitude**		
QN		**VG**	horizontal	RT8711	1
			inclined	T1463LM	2
			vertical	RT0976	3
			half-turned down	U0881BG	4
			turned down	F5DN3MA	5
			over turned	IR179DMR	6
32. (+)	**34. (*)**	**80-89**	**Head: diameter**		
QN		**MS/VG**	very small		1
			very small to small		2
			small	RT0976	3
			small to medium		4
			medium	BT0835, HA89	5
			medium to large		6
			large	F5DN3MA	7
			large to very large		8
			very large		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
33. (+)	**35. (*)**	**85-87**	**Head: shape of seed side**		
PQ		**VG**	strongly concave		1
			weakly concave	R5PG6MJ	2
			flat	RT8711	3
			weakly convex	HA89, R6ST2MI	4
			strongly convex	T0916LG	5
			deformed	TRC3398R	6
34.	**36.**	**99**	**Seed: size**		
QN		**MS/VG**	very small	PHA283	1
			very small to small		2
			small	TRC2342	3
			small to medium		4
			medium	HA89, OB724	5
			medium to large		6
			large	FT2603, Kisvárdai	7
			large to very large		8
			very large		9
35. (+)	**37. (*)**	**99**	**Seed: shape**		
PQ		**VG**	elongated	BT0835	1
			narrow ovoid	H11050R	2
			broad ovoid	F7AW1MOA, HA89	3
			rounded	KHEO777R, KHM4942R	4
36.	**38.**	**99**	**Seed: thickness relative to width**		
QN		**MS/VG**	very thin	RHA801	1
			thin		2
			medium	F7AW1MOA, FR83322	3
			thick	85C11R, F7AX2MA	4
			very thick	F76051	5

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
37. (+)	**39. (*)**	**99**	**Seed: colour**		
PQ		**VG**	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
G			black	HA89, T0954LM	9
38. (+)	**40. (*)**	**99**	**Seed: stripes on margin**		
QN		**VG**	none or very weak	T0954LM	1
			weak	OB724	2
G			strong	HA89, U0881BG	3
39. (+)	**41. (*)**	**99**	**Seed: stripes between margins**		
QN		**VG**	none or very weak	T0954LM	1
			weak	LGR27	2
G			strong	HA89, U0881BG	3
40.	**42. (*)**	**99**	**Seed: colour of stripes**		
PQ		**VG**	white	U0881BG	1
			brown	F1164LM	2
			grey	99D40R	3
			black		4

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 Explanations covering several characteristics

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

a) Observations should be made on fully developed leaves on the upper third of the plant.

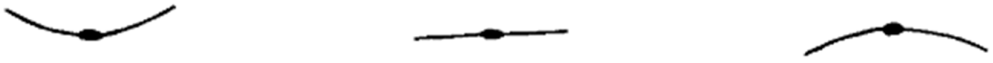
8.2 Explanations for individual characteristics

Ad. 4: Leaf: serration

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

Ad. 5: Leaf: profile in cross-section

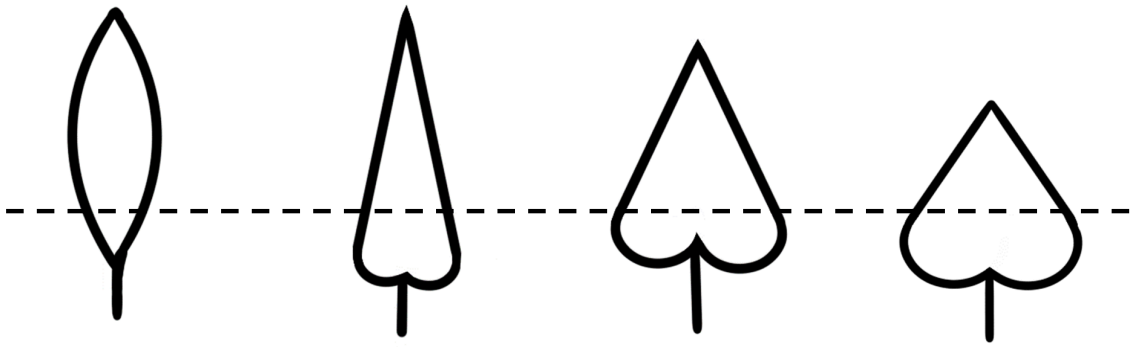
Cross-section:



1	3	5
strongly concave	flat	strongly convex

Ad. 6: Leaf: shape

Observations should be made on the distal part of the leaf.

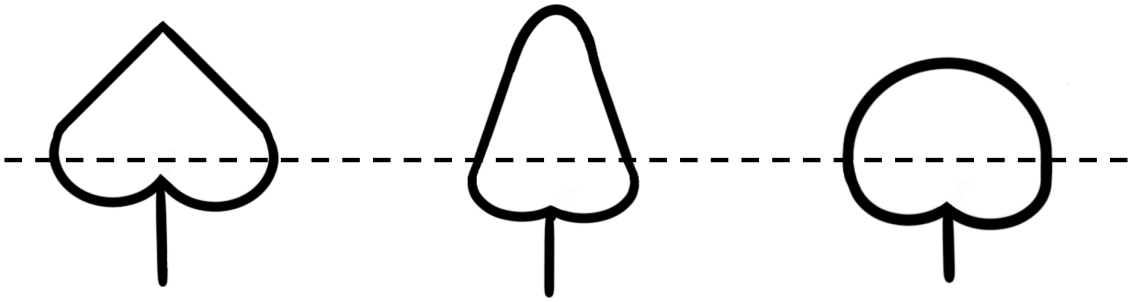


1
elliptic

2
very narrow triangular

3
narrow triangular

4
medium triangular



5
broad triangular

6
triangular to rounded

7
Rounded

Ad. 7: Leaf: lobes

1
absent or very small

3
small

5
medium

7
large

9
very large

Ad. 8: Leaf: parenchyma at base of lateral veins

1
none or very weak

2
weak

3
Strong

Ad. 9: Leaf: angle of lowest lateral veins

1
acute

2
right angle or nearly right angle

3
Obtuse

Ad. 11: Time of beginning of flowering

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

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

1
Erect

3
Horizontal

Ad. 13: Ray floret: profile

1
Flat

2
Rolled

3
twisted

4
strongly recurved

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

1
very narrow

2
narrow

3
broad

4
very broad

Ad. 17: Ray floret: colour

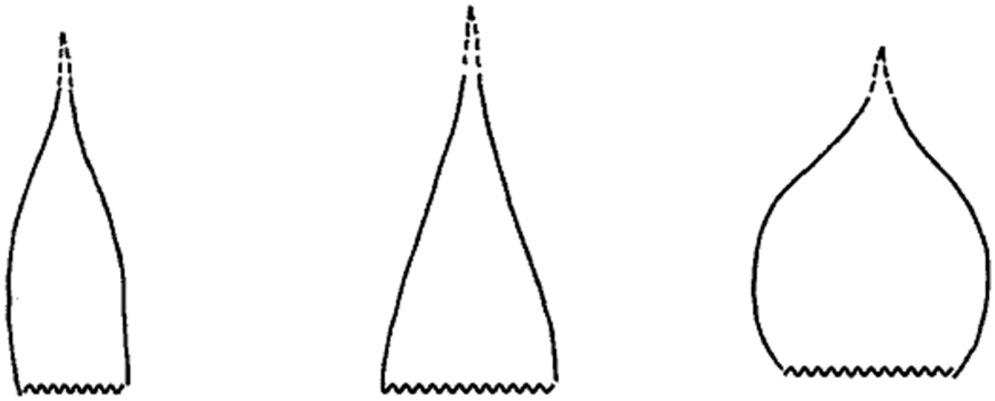
The ray floret colour is the colour with the largest surface area. In cases where the areas of the colour are too similar to reliability decide which colour has the largest area, the darker colour is to be observed.

Ad. 19: Disc floret: anthocyanin coloration of stigma

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

Ad. 21: Bract: shape

To be observed excluding the differentiated tip.



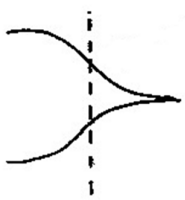
1
narrow acute

2
broad acute

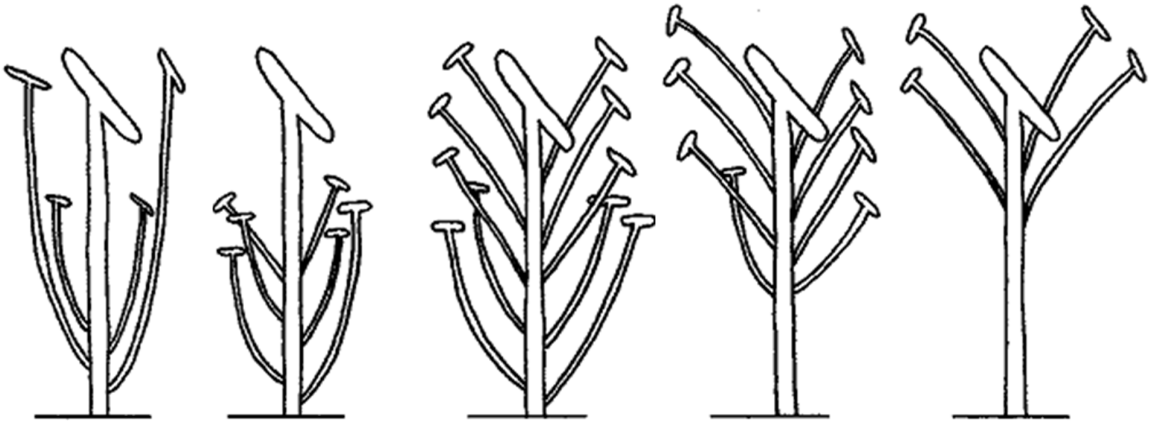
3
Rounded

Ad. 22: Bract: length of tip

Tip begins when the direction of curving changes.



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



1 only basal | 2 predominantly basal | 3 throughout | 4 predominantly apical | 5 only apical

Ad. 30: Stem: attitude

Observations should be made on the upper third of the stem below the head.

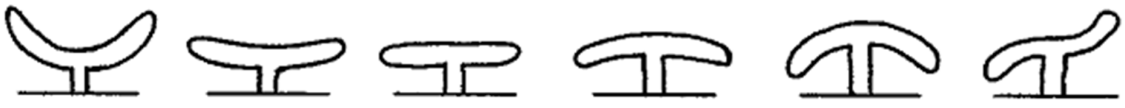
Ad. 31: Head: attitude

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

Ad. 32: Head: diameter

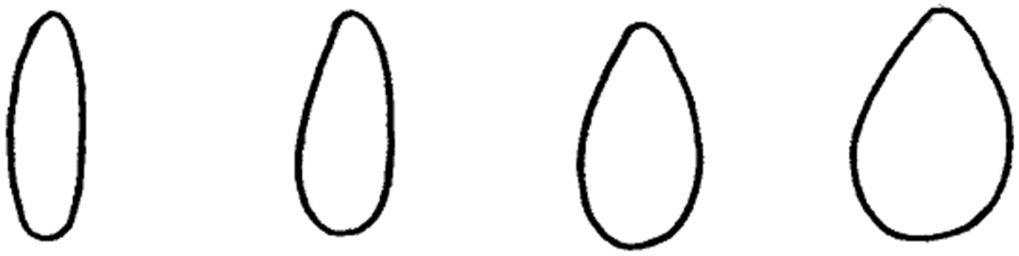
In the case of branching varieties, observations should be made on the central head.

Ad. 33: Head: shape of seed side



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

Ad. 35: Seed: shape



1
elongated

2
narrow ovoid

3
broad ovoid

4
rounded

Ad. 37: Seed: colour

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

Ad. 38: Seed: stripes on margin

Ad. 39: Seed: stripes between margins

8.3 Explanations on growth stages

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 development[1]	
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 [1](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 stigma visible)
65	Full flowering: disc florets in middle third of inflorescence in bloom (stamens and stigma visible)
67	Flowering declining: disc florets in inner third of inflorescence in bloom (stamens and stigma 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	Harvest product

ELECTROPHORESIS

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 has reconfirmed 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.

Characteristics Derived by Using Electrophoresis

	Characteristics	Example Varieties	Note
43. (+)	**Allele expression at locus Me1**		
	Genotype 2/2	HA 89	1
	Genotype 4/4	RHA 274	2
	Genotype 2/4	Florence	3
44. (+)	**Allele expression at locus Pgd1**		
	Genotype 2/2	RHA 274	1
	Genotype 4/4	HA 850	2
	Genotype 2/4	Santafe	3
45. (+)	**Allele expression at locus Pgi2**		
	Genotype 2/2	RHA 274	1
	Genotype 4/4	H559211	2
	Genotype 2/4	Santafe	3
46. (+)	**Allele expression at locus Shdh1**		
	Genotype 2/2	HA 89	1
	Genotype 4/4	RHA 856	2
	Genotype 2/4	Florence	3
47. (+)	**Allele expression at locus Pgm4**		
	Genotype 2/2	RHA 274	1
	Genotype 4/4	HA 89	2
	Genotype 2/4	Florence	3

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 Na_2)
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 ($MgCl_2$, $6H_2O$)
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). In order to improve the results on PGD, Polyvenyl-Pyrrolidone (PVP) of 2% (p/v) can be added to the solution.

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 $MgCl_2$ 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 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 or in moistened sterile sand, 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 300µl 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

Voltage should depend on the size of the gel, at a rate of 15 - 16 V/cm^2. Distance run by bromophenol blue (dye front) is also depending on the size of the gel, but the Duration of migration (time of migration) is a fixed value.

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.

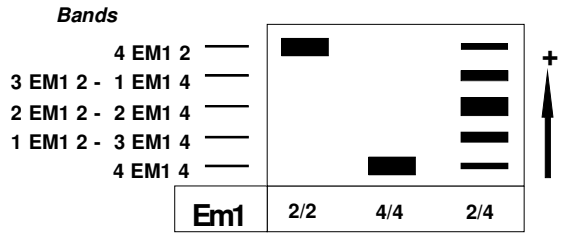
6. Recognition of the alleles encoding isoenzymes

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

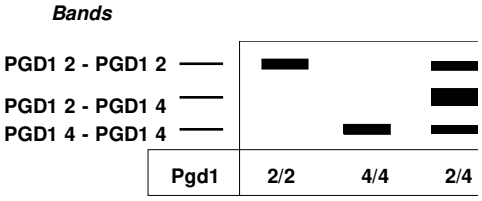


6.2 Recognition of the alleles encoding PGD

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



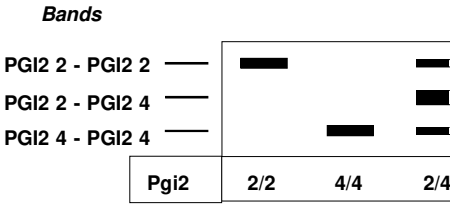
Two migration zones can be observed; only the slowest migrating bands are polymorphic.

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



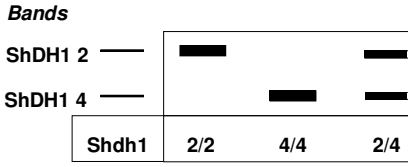
There are two migration zones; only the slowest migrating bands are scored.

6.4 Recognition of the alleles encoding ShDH

6.4.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Locus	Alleles
Shikimate dehydrogenase (S**h**DH)	Monomeric	Shdh1	2 4

6.4.2 Schematization of the zymogrammes

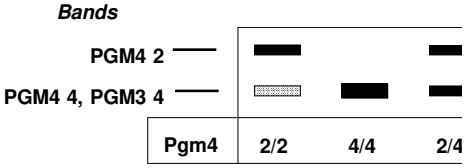


6.5 Recognition of the alleles encoding PGM

6.5.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Locus	Alleles
Phosphoglucomutase	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.

9. LITERATURE

SEMAE Formation, GEVES, 2024: Description des géniteurs et variétés de tournesol. (English, French, Spanish) SEMAE Formation, 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 Dakota, US, 1992

10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO/TQ-081/2 – *Helianthus annuus* L. - sunflower

Link to e-TQ:
https://online.plantvarieties.eu/backOfficeFormQuestions?viewFormId=17758&viewFormType=TQ&viewFormLang=EN&speciesIds=HEL01&status=1,2