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SUNFLOWER

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Helianthus annuus L.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

Alternative names:*

<i>Botanical name</i>	<i>English</i>	<i>French</i>	<i>German</i>	<i>Spanish</i>
<i>Helianthus annuus</i> L.	Common Sunflower	Tournesol, Soleil	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:

5,000 seeds for inbred lines
1 kg of seed for hybrid and open-pollinated varieties

In the case of hybrid varieties, an additional 5000 seeds 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 seeds 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 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

3.4 *Test Design*

- 3.4.1 Each test should be designed to result in a total of at least 40 plants, which should be divided between at least 2 replicates.
- 3.4.2 The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.5 *Additional Tests*

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 *Distinctness*

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

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

- (i) description of parent lines according to the Test Guidelines;
- (ii) check of the originality of the parent lines in comparison with the variety collection, based on the characteristics in Chapter 7, in order to identify similar parent lines;
- (iii) check of the originality of the hybrid formula in relation to the hybrids in the variety collection, taking into account the most similar lines; and
- (iv) assessment of the distinctness at the hybrid level for varieties with a similar formula.

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

4.1.2 Consistent Differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear Differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.1.4 Number of Plants or Parts of Plants to be Examined

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

4.1.5 Method of Observation

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

MG: single measurement of a group of plants or parts of plants

MS: measurement of a number of individual plants or parts of plants

VG: visual assessment by a single observation of a group of plants or parts of plants

VS: visual assessment by observation of individual plants or parts of plants

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

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

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

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

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

4.2 Uniformity

- 4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:
- 4.2.2 These Test Guidelines have been developed for the examination of seed-propagated varieties. For varieties with other types of propagation, the recommendations in the General Introduction and document TGP/13 "Guidance for new types and species" Section 4.5 "Testing Uniformity" should be followed.
- 4.2.3 The assessment of uniformity for open-pollinated varieties should be according to the recommendations for cross-pollinated varieties in the General Introduction.
- 4.2.4 The assessment of uniformity for hybrid varieties depends on the type of hybrid and should be according to the recommendations for hybrid varieties in the General Introduction.
- 4.2.5 Where the assessment of a hybrid variety involves the parent lines, the uniformity of the hybrid variety should, in addition to an examination of the hybrid variety itself, also be assessed by examination of the uniformity of its parent lines.
- 4.2.6 For the assessment of uniformity of inbred lines, a population standard of 2% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 36 plants, 2 off-types are allowed. In addition, the same population standard and acceptance probability should apply for the assessment of uniformity regarding out-crosses and isogenic male fertile plants in a male sterile line. For the assessment of uniformity of single hybrids, a population standard of 5% with an acceptance probability of at least 95% should be applied. In the case of a sample size of 36 plants, 4 off-types are allowed. For three-way hybrids and open-pollinated varieties, the variability within the variety should not exceed the variability of comparable varieties already known.

4.3 *Stability*

- 4.3.1 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.
- 4.3.2 Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.
- 4.3.3 Where appropriate, or in cases of doubt, the stability of a hybrid variety may, in addition to an examination of the hybrid variety itself, also be assessed by examination of the uniformity and stability of its parent lines.

5. Grouping of Varieties and Organization of the Growing Trial

- 5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.
- 5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.
- 5.3 The following have been agreed as useful grouping characteristics:
- (a) Leaf: intensity of green color (characteristic 2)
 - (b) Leaf: blistering (characteristic 3)
 - (c) Time of beginning of flowering (characteristic 11)
 - (d) Ray floret: color (characteristic 17)
 - (e) Disc floret: production of pollen (characteristic 22)
 - (f) Only inbred lines: Plant: natural height (characteristic 27)
 - (g) Only hybrids and open-pollinated varieties: Plant: natural height (characteristic 28)
 - (h) Plant: branching (characteristic 29)
 - (i) Seed: color (characteristic 39)
 - (j) Seed: stripes on margin (characteristic 40)
 - (k) Seed: stripes between margins (characteristic 41)
- 5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction and document TGP/9 "Examining Distinctness".

6. Introduction to the Table of Characteristics

6.1 *Categories of Characteristics*

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

6.2 *States of Expression and Corresponding Notes*

6.2.1 States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

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

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

6.3 *Types of Expression*

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 *Example Varieties*

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

6.5 Legend

		English	français	deutsch	español	Example Varieties 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) See Explanations on the Table of Characteristics in Chapter 8.1

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

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

	English		français	deutsch	español	Example Varieties Exemples Beispielsorten Variedades ejemplo	Note/ Nota
1.	QN	VG		10			
	Seedling: anthocyanin coloration of hypocotyl	Plantule : pigmentation anthocyanique de l'hypocotyle	Keimpflanze: Anthocyanfärbung des Hypokotyls	Plántula: pigmentación antocianica del hipocótulo			
	absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	T0954LM	1	
	weak	faible	gering	débil	OB724	2	
	medium	moyenne	mittel	media	TRC3285	3	
	strong	forte	stark	fuerte	F7AW1MOA	4	
	very strong	très forte	sehr stark	muy fuerte	Kisvárdai	5	
2. (*)	QN	VG	(a)	51-55			
	Leaf: intensity of green color	Feuille : intensité de la couleur verte	Blatt: Intensität der Grünfärbung	Hoja: intensidad del color verde			
	very light	très claire	sehr hell	muy clara	F5DN3MA, T0243HG	1	
	light	claire	hell	clara		2	
	medium	moyenne	mittel	media	H11050R	3	
	dark	foncée	dunkel	oscura		4	
	very dark	très foncée	sehr dunkel	muy oscura	13013	5	
3. (*)	QN	VG	(a)	51-55			
	Leaf: blistering	Feuille : gaufre	Blatt: Blasigkeit	Hoja: abullonado			
	absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	F5DN3MA	1	
	very weak to weak	très faible à faible	sehr gering bis gering	muy débil a débil		2	
	weak	faible	gering	débil	F7AX2JA, IR79DMR	3	
	weak to medium	faible à moyenne	gering bis mittel	débil a medio		4	
	medium	moyenne	mittel	medio	HA89, IB1088DMR	5	
	medium to strong	moyenne à forte	mittel bis stark	medio a fuerte		6	
	strong	forte	stark	fuerte	TRC2342	7	
	strong to very strong	forte à très forte	stark bis sehr stark	fuerte a muy fuerte		8	
	very strong	très forte	sehr stark	muy fuerte		9	

	English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
4. (*)	QN	VG	(+)	(a)	51-55			
	Leaf: serration	Feuille : denture	Blatt: Randeinschnitte	Hoja: serrado				
	isolated or very fine	isolée ou très fine	vereinzelt oder sehr fein	aislado o muy fino	99D40R			1
	very fine to fine	très fine à fine	sehr fein bis fein	muy fino a fino				2
	fine	fine	fein	fino	IR79DMR			3
	fine to medium	fine à moyenne	fein bis mittel	fino a medio				4
	medium	moyenne	mittel	medio	HA89, TRC2342			5
	medium to coarse	moyenne à grossière	mittel bis grob	medio a grosero				6
	coarse	grossière	grob	grosero	PB1458DMR			7
	coarse to very coarse	grossière à très grossière	grob bis sehr grob	grosero a muy grosero				8
	very coarse	très grossière	sehr grob	muy grosero				9
5.	QN	VG	(+)	(a)	53-55			
	Leaf: profile in cross-section	Feuille : profil en section transversale	Blatt: Profil im Querschnitt	Hoja: perfil en sección transversal				
	strongly concave	fortement concave	stark konkav	fuertemente cóncavo	RT9513			1
	weakly concave	faiblement concave	schwach konkav	débilmente cóncavo				2
	flat	plate	gerade	plano	PH5002R			3
	weakly convex	faiblement convexe	schwach konvex	débilmente convexo				4
	strongly convex	fortement convexe	stark konvex	fuertemente convexo				5
6.	PQ	VG	(+)	(a)	53-55			
	Leaf: shape	Feuille : forme	Blatt: Form	Hoja: forma				
	elliptic	elliptique	elliptisch	elíptica	FR810RM1			1
	very narrow triangular	triangulaire très étroite	sehr schmal dreieckig	triangular muy estrecha	FR81013			2
	narrow triangular	triangulaire étroite	schmal dreieckig	triangular estrecha	RT0976			3
	medium triangular	triangulaire moyenne	mittel dreieckig	triangular media	RT9513			4
	broad triangular	triangulaire large	breit dreieckig	triangular ancha	BT0835			5
	triangular to rounded	triangulaire à arrondie	dreieckig bis abgerundet	triangular a redondeada				6
	rounded	arrondie	abgerundet	redondeada				7

	English		français		deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
7. (*)	QN	VG	(+)	(a)	53-55			
	Leaf: lobes	Feuille : lobes	Blatt: Lappen	Hoja: lóbulos				
	absent or very small	absents ou très petits	fehlend oder sehr klein	ausentes o muy pequeños	37025			1
	very small to small	très petits à petits	sehr klein bis klein	muy pequeños a pequeños				2
	small	petits	klein	pequeños	T0954LM			3
	small to medium	petits à moyens	klein bis mittel	pequeños a medias				4
	medium	moyens	mittel	medios				5
	medium to large	moyens à grands	mittel bis groß	medios a grandes				6
	large	grands	groß	grandes	F6AH6MO, HA89			7
	large to very large	grands à très grands	groß bis sehr groß	grandes a muy grandes				8
	very large	très grands	sehr groß	muy grandes	RHA299			9
8.	QN	VG	(+)	(a)	53-55			
	Leaf: parenchyma at base of lateral veins	Feuille : parenchyme à la base des nervures latérales	Blatt: Parenchym an der Basis der untersten Seitennerven	Hoja: parénquima en la base de los nervios laterales				
	none or very weak	absent ou très faible	fehlend oder sehr gering	ausente o muy débil	T0954LM			1
	weak	faible	gering	débil	F7AW1MOA			2
	strong	fort	stark	fuerte	13013			3
9. (*)	QN	VG	(+)	(a)	53-55			
	Leaf: angle of lowest lateral veins	Feuille : angle des nervures latérales les plus basses	Blatt: Winkel der untersten Seitenadern	Hoja: ángulo de los nervios laterales inferiores				
	acute	aigu	spitz	agudo	T0860LM			1
	right angle or nearly right angle	droit ou presque droit	rechtwinklig oder fast rechtwinklig	ángulo recto o casi ángulo recto	F7AW1MOA			2
	obtuse	obtus	stumpf	obtuso	TFC3767B			3
10. (*)	QN	MS/VG		(a)	55-57			
	Leaf: size	Feuille : taille	Blatt: Größe	Hoja: tamaño				
	very small	très petite	sehr klein	muy pequeño				1
	very small to small	très petite à petite	sehr klein bis klein	muy pequeño a pequeño				2
	small	petite	klein	pequeño	PH5002R			3
	small to medium	petite à moyenne	klein bis mittel	pequeño a medio				4
	medium	moyenne	mittel	medio	LC1093, OB724			5
	medium to large	moyenne à grande	mittel bis groß	medio a grande				6
	large	grande	groß	grande	IA1169DMR			7
	large to very large	grande à très grande	groß bis sehr groß	grande a muy grande				8
	very large	très grande	sehr groß	muy grande				9

	English		français		deutsch		español		Example Varieties Exemples Beispielssorten Variedades ejemplo		Note/ Nota
11. (*)	QN	MG/MS	(+)		61						
	Time of beginning of flowering	Époque du début de la floraison	Zeitpunkt des Blühbeginns	Época de inicio de la floración							
	very early	très précoce	sehr früh	muy temprana	PHA283						1
	very early to early	très précoce à précoce	sehr früh bis früh	muy temprana a temprana							2
	early	précoce	früh	temprana	T0860LM						3
	early to medium	précoce à moyenne	früh bis mittel	temprana a media							4
	medium	moyenne	mittel	media	H11050R, RHA274						5
	medium to late	moyenne à tardive	mittel bis spät	media a tardía							6
	late	tardive	spät	tardía	RT7710						7
	late to very late	tardive à très tardive	spät bis sehr spät	tardía a muy tardía							8
	very late	très tardive	sehr spät	muy tardía	Kisvárdai, LGR27						9
12.	QN	VG	(+)		63-65						
	Ray floret: attitude of base in relation to head	Fleur ligulée : port de la base par rapport au capitule	Zungenblüte: Haltung der Basis im Verhältnis zum Kopf	Flor ligulada: porte de la base en relación con el capítulo							
	erect	dressé	aufgerichtet	erecto	T0833HG						1
	semi-erect	demi-dressé	halbaufgerichtet	semierecto							2
	horizontal	horizontal	waagrecht	horizontal	T0954LM						3
13.	PQ	VG	(+)		63-65						
	Ray floret: profile	Fleur ligulée : profil	Zungenblüte: Profil	Flor ligulada: perfil							
	flat	plat	eben	plano	HA89, IR79DMR						1
	rolled	enroulé	gerollt	enrollado	PH5002R						2
	twisted	torsadé	gedreht	torcido	F5DN3MA						3
	strongly recurved	fortement recourbé	stark gebogen	fuertemente recurvado							4
14.	QN	VG			63-65						
	Flower: density of ray florets	Fleur : densité des fleurs ligulées	Blüte: Dichte der Zungenblüten	Flor: densidad de las flores liguladas							
	very sparse	très lâche	sehr locker	muy laxa	T0954LM						1
	sparse	lâche	locker	laxa							2
	medium	moyenne	mittel	media	99D40R, HA89						3
	dense	dense	dicht	densa							4
	very dense	très dense	sehr dicht	muy densa	OB724						5

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
15.	QN	MS/VG		63-65		
	Ray floret: length	Fleur ligulée : longueur	Zungenblüte: Länge	Flor ligulada: longitud		
	very short	très courte	sehr kurz bis kurz	muy corta	BT0835	1
	short	courte	kurz	corta		2
	medium	moyenne	mittel	media	SF9074MA	3
	long	longue	lang	larga		4
	very long	très longue	sehr lang bis sehr lang	muy larga	T0954LM	5
16.	QN	MS/VG	(+)	63-65		
	Ray floret: width in relation to length	Fleur ligulée : largeur par rapport à la longueur	Zungenblüte: Breite im Verhältnis zur Länge	Flor ligulada: anchura en relación con la longitud		
	very narrow	très étroite	sehr schmal	muy estrecha	T0954LM	1
	narrow	étroite	schmal	estrecha	HA850, OB724	2
	broad	large	breit	ancha		3
	very broad	très large	sehr breit	muy ancha		4
17. (*)	PQ	VG	(+)	63-65		
	Ray floret: color	Fleur ligulée : couleur	Zungenblüte: Farbe	Flor ligulada: color		
	yellowish white	blanc jaunâtre	gelblichweiß	blanco amarillento	RHA381	1
	light yellow	jaune clair	hellgelb	amarillo claro	F7AW1MOA	2
	medium yellow	jaune moyen	mittelgelb	amarillo medio	RT7710	3
	orange yellow	jaune orange	orangegelb	amarillo anaranjado	U0881BG	4
	orange	orange	orange	naranja	OB724, P211R	5
	purple	pourpre	purpurn	púrpura		6
	reddish brown	brun rougeâtre	rötlichbraun	marrón rojizo		7
18.	QL	VG	(+)	63-65		
	Disc floret: anthocyanin coloration of pappus	Fleuron : pigmentation anthocyanique du pappus	Röhrenblüte: Anthocyanfärbung des Pappus	Flósculo: pigmentación antociánica del papus		
	absent	absente	fehlend	ausente	F7EW4IMO	1
	present	présente	vorhanden	presente	OKD4447R, TRC2342	9
19.	PQ	VG		63-65		
	Disc floret: color	Fleuron : couleur	Röhrenblüte: Farbe	Flósculo: color		
	yellow	jaune	gelb	amarillo	STR226, TRC2342	1
	orange	orange	orange	naranja	F7AW1MOA, HA89	2
	purple	pourpre	purpurn	púrpura		3

	English		français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
20.	QL	VG	(+)	63-65			
	Disc floret: anthocyanin coloration of anthers		Fleuron : pigmentation anthocyanique des anthères	Röhrenblüte: Anthocyanfärbung der Antheren	Flósculo: pigmentación antociánica de las anteras		
	absent		absente	fehlend	ausente	R4NO4MJ	1
	present		présente	vorhanden	presente	R5XY3MJS	9
21.	QN	VG	(+)	63-65			
	Disc floret: anthocyanin coloration of stigma		Fleuron : pigmentation anthocyanique du stigmate	Röhrenblüte: Anthocyanfärbung der Narbe	Flósculo: pigmentación antociánica del estigma		
	absent or very weak		absente ou très faible	fehlend oder sehr gering	ausente o muy débil	SF9074MA	1
	weak		faible	gering	débil	RT7710	2
	medium		moyenne	mittel	media	R6ST2MI, TRC2342	3
	strong		forte	stark	fuerte	F7AW1MOA	4
	very strong		très forte	sehr stark	muy fuerte	Kisvárdai	5
22. (*)	QL	VG		63-65			
	Disc floret: production of pollen		Fleuron: production de pollen	Scheibenblüte: Pollenproduktion	Flósculo: producción de polen		
	absent		absente	fehlend	ausente	F7AW1MOA, HA89	1
	present		présente	vorhanden	presente	IR79DMR, RHA274	9
23.	PQ	VG	(+)	63-65			
	Bract: shape		Bractée : forme	Hüllblatt: Form	Bráctea: forma		
	narrow acute		aiguë étroite	schmal spitz	aguda estrecha	T0954LM	1
	broad acute		aiguë large	breit spitz	aguda ancha	IR79DMR	2
	rounded		arrondie	abgerundet	redondeada	IB1088DMR	3
24.	QN	MS/VG	(+)	63-65			
	Bract: length of tip		Bractée : longueur de l'extrémité	Hüllblatt: Länge der Spitze	Bráctea: longitud de la punta		
	very short		très courte	sehr kurz	muy corta	IB1088DMR	1
	short		courte	kurz	corta		2
	medium		moyenne	mittel	media	HA89, T0954LM	3
	long		longue	lang	larga		4
	very long		très longue	sehr lang	muy larga	U0881BG	5

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
25.	QN	VG	63-65			
	Bract: intensity of green color of outer side	Bractée : intensité de la couleur verte de la face externe	Hüllblatt: Intensität der Grünfärbung der Außenseite	Bráctea: intensidad del color verde de la cara externa		
	light	claire	hell	clara	T0243HG	1
	medium	moyenne	mittel	media	T0954LM	2
	dark	foncée	dunkel	oscura	RT8711	3
26.	QN	VG	69-73			
	Bract: attitude in relation to head	Bractée : port par rapport au capitule	Hüllblatt: Haltung im Verhältnis zum Korb	Bráctea: porte en relación con n el capítulo		
	not adpressed or very slightly adpressed	non apprimé ou très faiblement apprimé	nicht anliegend oder sehr leicht anliegend	no adpreso o muy ligeramente adpreso	HA89, RT0976	1
	slightly adpressed	légèrement apprimé	leicht anliegend	ligeramente adpreso	F7AW1MOA	2
	strongly adpressed	fortement apprimé	stark anliegend	fuertemente adpreso	RT9513	3
27. (*)	QN	MS	69-73			
	<u>Only inbred lines:</u> Plant: natural height	<u>Seulement pour les lignées :</u> Plante : hauteur naturelle	<u>Nur Inzuchtlinien:</u> Pflanze: natürliche Höhe	<u>Sólo variedades endógamas:</u> Planta: altura natural		
	very short	très basse	sehr niedrig	muy baja	FR810RM1	1
	very short to short	très basse à basse	sehr niedrig bis niedrig	muy baja a baja		2
	short	basse	niedrig	baja	OB724	3
	short to medium	basse à moyenne	niedrig bis mittel	baja a media		4
	medium	moyenne	mittel	media	U0881BG	5
	medium to tall	moyenne à haute	mittel bis hoch	media a alta		6
	tall	haute	hoch	alta	R6ST2MI	7
	tall to very tall	haute à très haute	hoch bis sehr hoch	alta a muy alta		8
	very tall	très haute	sehr hoch	muy alta	31G03	9

	English	français	deutsch	español	Example Varieties Exemples Beispielsorten Variedades ejemplo	Note/ Nota
28. (*)	QN	MS		69-73		
	<u>Only hybrids and open-pollinated varieties:</u> Plant: natural height	<u>Seulement pour les hybrides et les variétés à fécondation libre :</u> Plante : hauteur naturelle	<u>Nur Hybriden und freiabblühende Sorten:</u> Pflanze: natürliche Höhe	<u>Sólo híbridos y variedades de polinización libre:</u> Planta: altura natural		
	very short	très basse	sehr niedrig	muy baja	Antonil	1
	very short to short	très basse à basse	sehr niedrig bis niedrig	muy baja a baja		2
	short	basse	niedrig	baja	GK Milia	3
	short to medium	basse à moyenne	niedrig bis mittel	baja a media		4
	medium	moyenne	mittel	media	Sumiko	5
	medium to tall	moyenne à haute	mittel bis hoch	media a alta		6
	tall	haute	hoch	alta	Marley	7
	tall to very tall	haute à très haute	hoch bis sehr hoch	alta a muy alta		8
	very tall	très haute	sehr hoch	muy alta	Kisvárdai	9
29. (*)	QL	VG		69-89		
	Plant: branching	Plante : ramification	Pflanze: Verzweigung	Planta: ramificación		
	absent	absente	fehlend	ausente	HA89, OB724	1
	present	présente	vorhanden	presente	RHA274, T0954LM	9
30. (*)	PQ	VG	(+)	69-89		
	<u>Only varieties with Plant: branching:</u> present: Plant: position of branching	<u>Seulement les variétés avec Plante : ramification :</u> présente : Plante : position de la ramification	<u>Nur Sorten mit Pflanze: Verzweigung: vorhanden:</u> Pflanze: Position der Verzweigung	<u>Solo variedades con Planta: ramificación: presente:</u> Planta: posición de la ramificación		
	only basal	uniquement basale	nur basal	sólo basal		1
	predominantly basal	prédominance basale	überwiegend basal	predominantemente basal		2
	throughout	partout	überall	total	H11050R	3
	predominantly apical	prédominance apicale	überwiegend apikal	predominantemente apical	RHA274, T0954LM	4
	only apical	uniquement apicale	nur apikal	sólo apical	TRC2342	5
31.	QN	VG		69-89		
	<u>Only varieties with Plant: branching:</u> present: Plant: position of highest lateral head to central head	<u>Seulement les variétés avec Plante : ramification :</u> présente : Plante : position du capitule latéral le plus haut par rapport au capitule central	<u>Nur Sorten mit Pflanze: Verzweigung: vorhanden:</u> Pflanze: Position des höchsten Seitenkorbes zum Hauptkorb	<u>Solo variedades con Planta: ramificación: presente:</u> Planta: posición natural del capítulo lateral más alto, en relación con el capítulo central		
	below	au-dessous	unterhalb	debajo	PH5004R	1
	same level	au même niveau	gleiche Höhe	al mismo nivel	T0954LM	2
	above	au-dessus	oberhalb	encima	99D40R	3

	English		français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
32. (*)	QN	VG	(+)	80-89			
	Stem: attitude		Tige : port	Stängel: Haltung	Tallo: porte		
	straight		droit	gerade	recto	U0881BG	1
	slightly curved		légèrement arqué	leicht gebogen	ligeramente arqueado		2
	strongly curved		fortement arqué	stark gebogen	fuertemente arqueado	F7EW2MIA	3
33. (*)	QN	VG	(+)	80-89			
	Head: attitude		Capitule : port	Kopf: Haltung	Capítulo: porte		
	horizontal		horizontal	waagrecht	horizontal	RT8711	1
	inclined		incliné	geneigt	inclinado		2
	vertical		vertical	vertikal	vertical	RT0976	3
	half-turned down		demi-renversé	halbüberhängend	semiinvertido	U0881BG	4
	turned down		renversé	überhängend	invertido	F5DN3MA	5
	over turned		retourné	zurückgebogen	retorcido		6
34. (*)	QN	MS/VG	(+)	80-89			
	Head: diameter		Capitule : diamètre	Korb: Durchmesser	Capítulo: diámetro		
	very small		très petit	sehr klein	muy pequeño		1
	very small to small		très petit à petit	sehr klein bis klein	muy pequeño a pequeño		2
	small		petit	klein	pequeño	RT0976	3
	small to medium		petit à moyen	klein bis mittel	pequeño a medio		4
	medium		moyen	mittel	medio	BT0835, HA89	5
	medium to large		moyen à grand	mittel bis groß	medio a grande		6
	large		grand	groß	grande	F5DN3MA	7
	large to very large		grand à très grand	groß bis sehr groß	grande a muy grande		8
	very large		très grand	sehr groß	muy grande		9
35. (*)	PQ	VG	(+)	85-87			
	Head: shape of seed side		Capitule : forme de la face portant les semences	Korb: Form der Kornseite	Capítulo: forma de la parte de la semilla		
	strongly concave		fortement concave	stark konkav	fuertemente cóncava		1
	weakly concave		faiblement concave	schwach konkav	débilmente cóncava	R5PG6MJ	2
	flat		plate	gerade	plana	RT8711	3
	weakly convex		faiblement convexe	schwach konvex	débilmente convexe	HA89, R6ST2MI	4
	strongly convex		fortement convexe	stark konvex	fuertemente convexe	T0916LG	5
	deformed		difforme	verformt	deformada	TRC3398R	6

	English	français	deutsch	español	Example Varieties Exemples Beispielsorten Variedades ejemplo	Note/ Nota
36.	QN	MS/VG		99		
	Seed: size	Graine : taille	Korn: Größe	Semilla: tamaño		
	very small	très petite	sehr klein	muy pequeño	PHA283	1
	very small to small	très petite à petite	sehr klein bis klein	muy pequeño a pequeño		2
	small	petite	klein	pequeño	TRC2342	3
	small to medium	petite à moyenne	klein bis mittel	pequeño a medio		4
	medium	moyenne	mittel	medio	HA89, OB724	5
	medium to large	moyenne à grande	mittel bis groß	medio a grande		6
	large	grande	groß	grande	FT2603, Kiszárdai	7
	large to very large	grande à très grande	groß bis sehr groß	grande a muy grande		8
	very large	très grande	sehr groß	muy grande		9
37. (*)	PQ	VG	(+)	99		
	Seed: shape	Graine : forme	Korn: Form	Semilla: forma		
	elongated	allongée	langezogen	alargada	BT0835	1
	narrow ovoid	ovoïde étroite	schmal eiförmig	ovoide estrecha	H11050R	2
	broad ovoid	ovoïde large	breit eiförmig	ovoide ancha	F7AW1MOA, HA89	3
	rounded	arrondie	abgerundet	redondeada		4
38.	QN	MS/VG		99		
	Seed: thickness relative to width	Graine : épaisseur par rapport à la largeur	Korn: Dicke im Verhältnis zur Breite	Semilla: grosor con relación a la anchura		
	very thin	très mince	sehr dünn	muy delgado	RHA801	1
	thin	mince	dünn	delgado		2
	medium	moyenne	mittel	medio	F7AW1MOA, FR83322	3
	thick	épaisse	dick	grueso	85C11R, F7AX2MA	4
	very thick	très épaisse	sehr dick	muy grueso		5
39. (*)	PQ	VG	(+)	99		
	Seed: color	Graine : couleur	Korn: Farbe	Semilla: color		
	white	blanc	weiß	blanco	Labud	1
	purple	pourpre	purpurn	púrpura		2
	light brown	brun clair	hellbraun	marrón claro	IR79DMR	3
	medium brown	brun moyen	mittelbraun	marrón medio	H11050R	4
	dark brown	brun foncé	dunkelbraun	marrón oscuro	B0644LM	5
	light grey	gris clair	hellgrau	gris claro	RW666IMI	6
	medium grey	gris moyen	mittelgrau	gris medio	RT9513	7
	dark grey	gris foncé	dunkelgrau	gris oscuro		8
	black	noir	schwarz	negro	HA89, T0954LM	9

	English		français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
40. (*)	QN	VG	(+)	99			
	Seed: stripes on margin	Graine : stries sur le bord	Korn: Streifen am Rand	Semilla: rayas en el borde			
	none or very weak	aucunes ou très faibles	keine oder sehr schwach	ausentes o muy débiles	T0954LM		1
	weak	faibles	schwach	débiles	OB724		2
	strong	fortes	stark	fuertes	HA89, U0881BG		3
41. (*)	QN	VG	(+)	99			
	Seed: stripes between margins	Graine : stries entre les bords	Korn: Streifen zwischen den Rändern	Semilla: rayas entre los bordes			
	none or very weak	aucunes ou très faibles	keine oder sehr schwach	ausentes o muy débiles	T0954LM		1
	weak	faibles	schwach	débiles	LGR27		2
	strong	fortes	stark	fuertes	HA89, U0881BG		3
42. (*)	PQ	VG		99			
	Seed: color of stripes	Graine : couleur des stries	Korn: Farbe der Streifen	Semilla: color de las rayas			
	white	blanc	weiß	blanco	U0881BG		1
	brown	brun	braun	marrón	F1164LM		2
	grey	gris	grau	gris	99D40R		3
	black	noir	schwarz	negro			4

8. Explanations on the Table of Characteristics

8.1 *Explanations covering several characteristics*

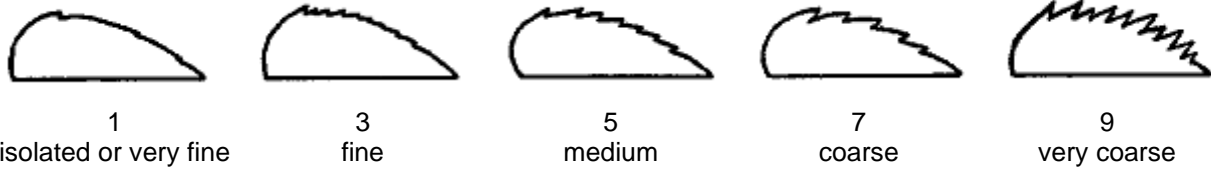
Unless otherwise indicated, observations should be made on the main stem.

Characteristics containing the following key in 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.

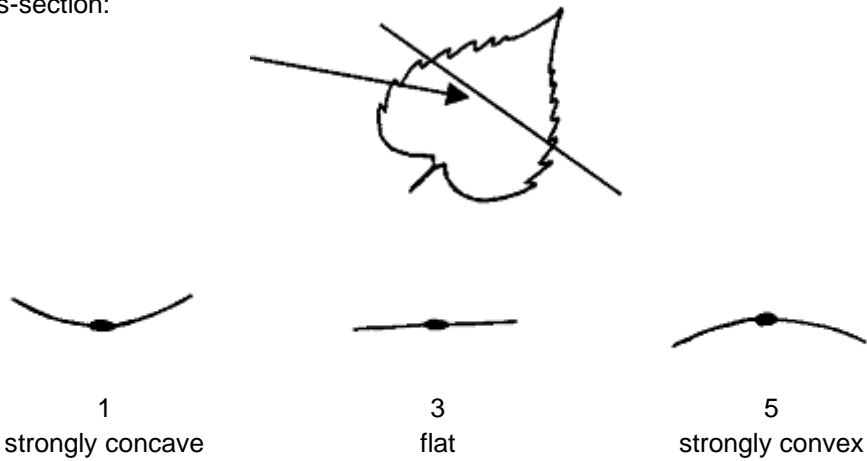
8.2 *Explanations for individual characteristics*

Ad. 4: Leaf: serration



Ad. 5: Leaf: profile in cross-section

Cross-section:



Ad. 6: Leaf: shape

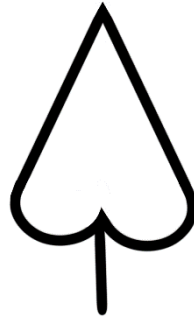
Observations should be made on the distal part of the leaf.



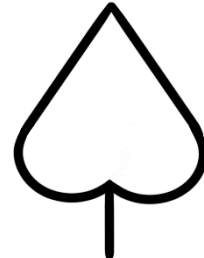
1
elliptic



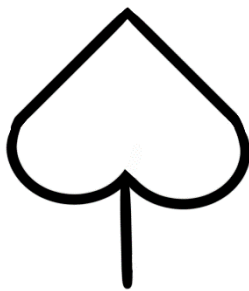
2
very narrow triangular



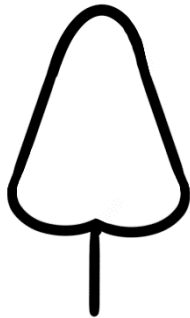
3
narrow triangular



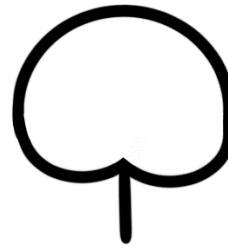
4
medium triangular



5
broad triangular



6
triangular to rounded



7
rounded

Ad. 7: Leaf: lobes



1
absent or very small



3
small



5
medium

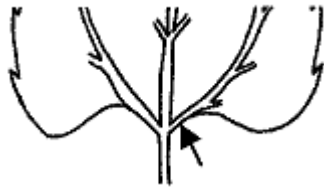


7
large

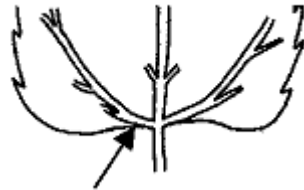


9
very large

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



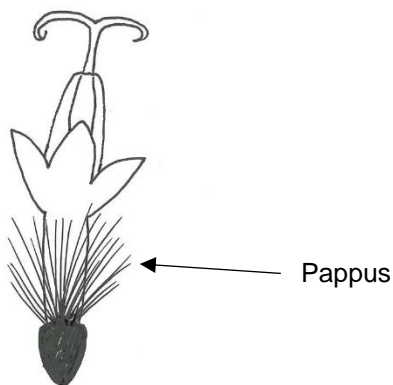
4
very broad

Ad. 17: Ray floret: color

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

Ad. 18: Disc floret: anthocyanin coloration of pappus

Observations should be made on the inner third of the disc.



Ad. 20: Disc floret: anthocyanin coloration of anthers

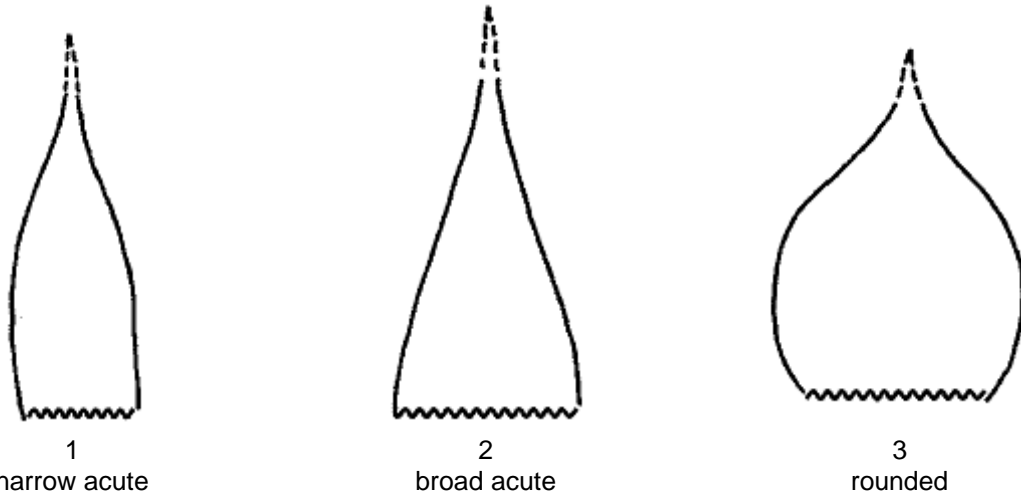
Observation should be made on the stigma just after the pollen appears at the top of the anthers.

Ad. 21: Disc floret: anthocyanin coloration of stigma

See Ad. 20

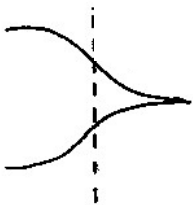
Ad. 23: Bract: shape

To be observed excluding the differentiated tip.

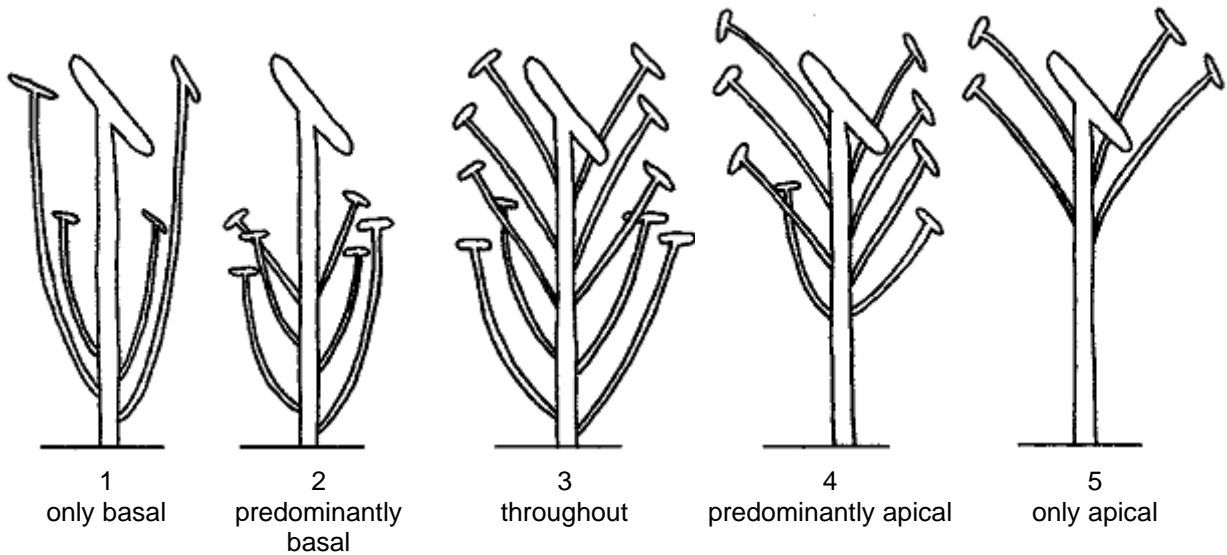


Ad. 24: Bract: length of tip

Tip begins where the direction of curving changes.



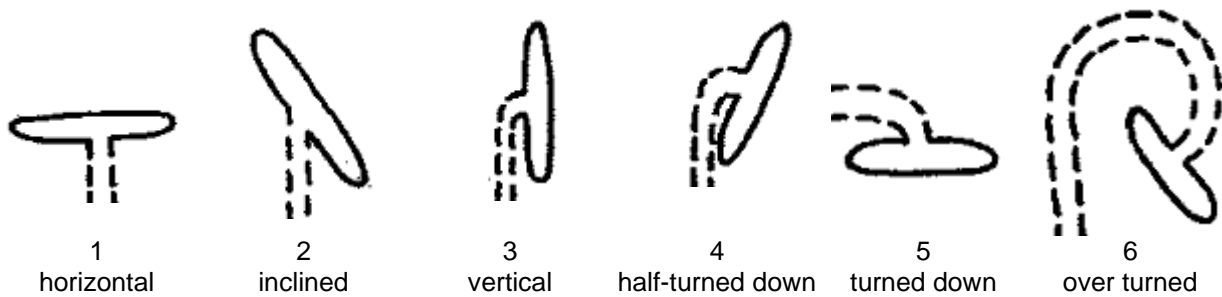
Ad. 30: Only varieties with Plant: branching: present: Plant: position of branching



Ad. 32: Stem: attitude

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

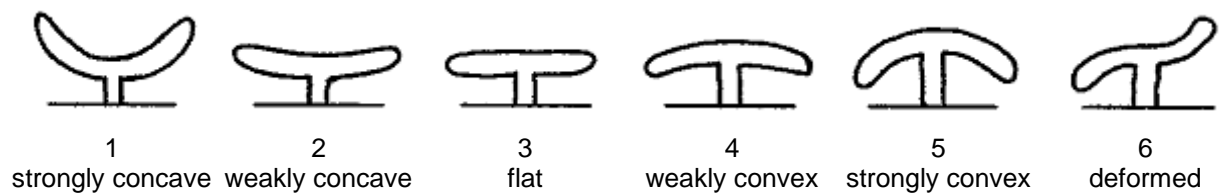
Ad. 33: Head: attitude



Ad. 34: Head: diameter

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

Ad. 35: Head: shape of seed side



Ad. 37: Seed: shape



1
elongated



2
narrow ovoid



3
broad ovoid



4
rounded

Ad. 39: Seed: color

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

Ad. 40: Seed: stripes on margin



Ad. 41: Seed: stripes between margins



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

Code Description

Principal growth stage 0: Germination

- 00 Dry seed (achene)
- 01 Beginning of seed imbibition
- 03 Seed imbibition complete
- 05 Radicle emerged from seed
- 06 Radicle elongated, root hairs developing
- 07 Hypocotyl with cotyledons emerged from seed
- 08 Hypocotyl with cotyledons growing towards soil surface
- 09 Emergence: cotyledons emerge through soil surface

Principal growth stage 1: Leaf development¹

- 10 Cotyledons completely unfolded
- 12 2 leaves (first pair) unfolded
- 14 4 leaves (second pair) unfolded
- 15 5 leaves unfolded
- 16 6 leaves unfolded
- 17 7 leaves unfolded
- 18 8 leaves unfolded
- 19 9 or more leaves unfolded

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 of anthocarp 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, 2000: Description des géniteurs et variétés de tournesol. (English, French, Spanish)
ASFIS, 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

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	<input type="text" value="Helianthus annuus L."/>
1.2	Common name	<input type="text" value="Sunflower"/>
2. Applicant		
	Name	<input type="text"/>
	Address	<input type="text"/>
	Telephone No.	<input type="text"/>
	Fax No.	<input type="text"/>
	E-mail address	<input type="text"/>
	Breeder (if different from applicant)	<input type="text"/>
3. Proposed denomination and breeder's reference		
	Proposed denomination (if available)	<input type="text"/>
	Breeder's reference	<input type="text"/>

#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.

4.2 Method of propagating the variety

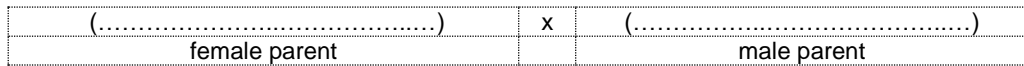
4.2.1 Seed-propagated varieties

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

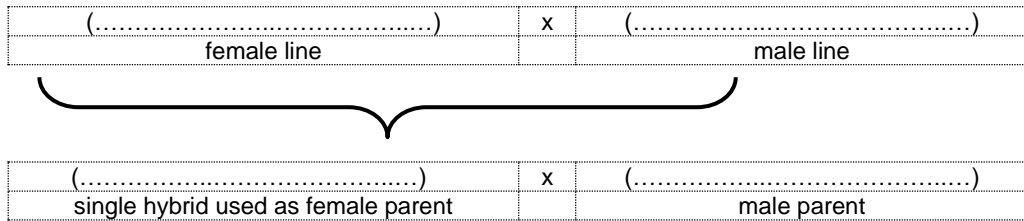
4.2.2 Other (Please provide details) []

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



Three-Way Hybrid



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

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.4 Ray floret: color (17)		
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 Disc floret: production of pollen (22)		
absent	F7AW1MOA, HA89	1 []
present	IR79DMR, RHA274	9 []
5.6 <u>Only inbred lines:</u> Plant: natural height (27)		
very short	FR810RM1	1 []
very short to short		2 []
short	OB724	3 []
short to medium		4 []
medium	U0881BG	5 []
medium to tall		6 []
tall	R6ST2MI	7 []
tall to very tall		8 []
very tall	31G03	9 []
5.7 <u>Only hybrids and open-pollinated varieties:</u> Plant: natural height (28)		
very short	Antonil	1 []
very short to short		2 []
short	GK Milia	3 []
short to medium		4 []
medium	Sumiko	5 []
medium to tall		6 []
tall	Marley	7 []
tall to very tall		8 []
very tall	Kisvárdai	9 []
5.8 Plant: branching (29)		
absent	HA89, OB724	1 []
present	RHA274, T0954LM	9 []

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.9 Seed: color (39)		
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 Seed: stripes on margin (40)		
none or very weak	T0954LM	1 []
weak	OB724	2 []
strong	HA89, U0881BG	3 []
5.11 Seed: stripes between margins (41)		
none or very weak	T0954LM	1 []
weak	LGR27	2 []
strong	HA89, U0881BG	3 []

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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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
<i>Example</i>	<i>Time of beginning of flowering</i>	<i>early</i>	<i>late</i>
Comments:			

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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#7. Additional information which may help in the examination of the variety

7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

Yes [] No []

(If yes, please provide details)

7.2 Are there any special conditions for growing the variety or conducting the examination?

Yes [] No []

(If yes, please provide details)

7.3 Other information

(1) Use

(a) oil and cake []

(b) birds consumption []

(c) direct human consumption (hulling type) []

(d) direct human consumption (confectionary) []

(e) other use (please specify) []

(2) Resistance to pests and diseases

(a) Downy mildew (specify the races) []

(b) Broomrape (specify 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 []

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

Additional Useful Explanations

	<u>TABLE OF CONTENTS</u>	<u>PAGE</u>
Part I	Introduction	2
Part II	Characteristics derived by using electrophoresis	3
Part III	Description of the method to be used	5

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 MgCl₂ solution (4.3.1.6.)
17.5 mg NADP

4.3.2.2 PGD + PGI staining solution

100 ml 0.1 M Tris HCl, pH 7.5 (4.3.1.1. diluted 1 in 10)
100 mg D-Fructose 6-phosphate Na₂ salt
60 mg 6-Phosphogluconic acid Na₃ salt
10 mg NADP
1 ml MTT solution (4.3.1.3.)
1.5 ml PMS solution (4.3.1.5.)
1 ml MgCl₂ solution (4.3.1.6.)
40 units of Glucose-6-phosphate dehydrogenase (SIGMA G 5885)

To stain PGI only, do not include 6-phosphogluconic acid.

To stain PGD only, do not include either fructose 6-phosphate disodium salt or glucose 6-phosphate dehydrogenase.

4.3.2.3 ShDH staining solution

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

4.3.2.4 PGM staining solution

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

5. Procedure

5.1. Enzyme extraction

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

The extracts can be stored at -30°C or at -80°C.

5.2 Preparation of the gel

Prepare the gels the day before migration.

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

5.3 Electrophoresis

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

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

The enzyme extracts are thawed from 5.1, and absorbed on a filter paper wick (1.5 mm x 20 mm, Whatman N° 3).

The wicks are inserted into the gel, tightly against the first slit.

One wick soaked with bromophenol blue solution (4.2.2.3) (migration dye marker) is placed on each side of the gel.

The gel slice is cautiously replaced. Each gel is covered with polyethylene film.

The two gels, with the extracts on the cathodal side, are placed on the two electrode buffer tanks, in a refrigerated cabinet at 4°C.

The electrophoresis is carried out at 4°C, towards the anode. After 15 min of migration at the first voltage, the wicks are removed and the voltage is increased. Constant voltage should be maintained during each phase.

The electrophoretic conditions are indicated in the following table.

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

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

5.4 Enzyme staining

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

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

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

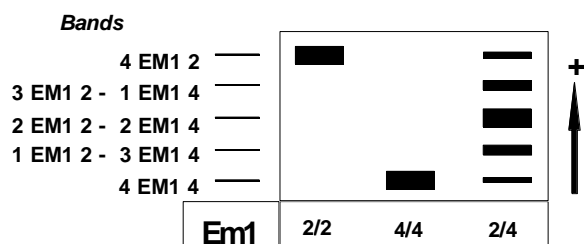
6. Recognition of the alleles encoding isoenzymes

6.1 Recognition of the alleles encoding ME

6.1.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Locus	Alleles
Malic enzyme (ME)	Tetrameric	Me1	2 4

6.1.2 Schematization of the zymogrammes

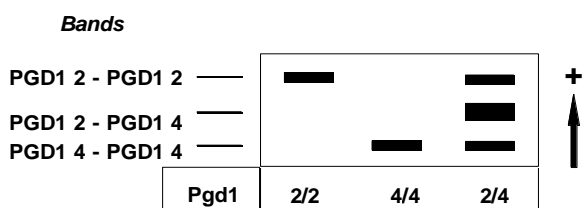


6.2 Recognition of the alleles encoding PGD

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



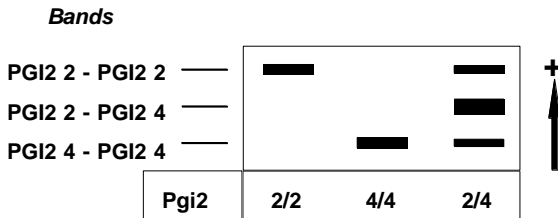
Two migration zones can be observed; only the slowest migrating bands are polymorphic.

6.3 Recognition of the alleles encoding PGI

6.3.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Locus	Alleles
Phosphoglucosomerase (PGI)	Dimeric	Pgi2	2 4

6.3.2 Schematization of the zymogrammes



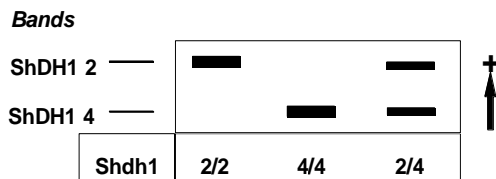
There are two migration zones; only the slowest migrating bands are scored.

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

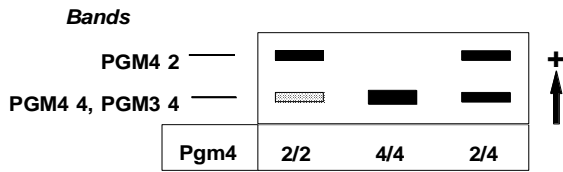


6.5 Recognition of the alleles encoding PGM

6.5.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Locus	Alleles
Phosphoglucomutase	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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