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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

MAIZE UPOV Code: ZEAAA_MAY <i>Zea mays</i> 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>Zea mays</i> L.	Maize, Corn	Maïs	Mais	Maíz

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 *Zea mays* 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:

1,500 grains for inbred lines;
1 kg for hybrids and open-pollinated varieties.

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*

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

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*

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.

3.3.1 Stage of development for the assessment

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

3.3.2 Type of observation

The recommended method of observing the characteristic is indicated by the following key in the second column of the Table 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

3.4 *Test Design*

Each test should be designed to result in a total of at least 40 plants in the case of inbred lines and single hybrids and 60 plants in the case of other hybrids and open-pollinated varieties. Each test should be divided between at least 2 replicates.

3.5 *Number of Plants / Parts of Plants to be Examined*

3.5.1 Inbred lines and single hybrids: All observations on single plants (MS) should be made on 10 plants or parts taken from each of 10 plants and all other observations made on all plants in the test.

3.5.2 Other types of hybrids: All observations on single plants (MS) should be made on 20 plants or parts taken from each of 20 plants and all other observations made on all plants in the test.

3.5.3 Open-pollinated varieties: All observations on single plants (MS) should be made on 40 plants or parts taken from each of 40 plants and all other observations made on all plants in the test.

3.6 *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, 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 Test Guidelines;
- (ii) check of the originality of the parental lines in comparison with the reference collection, based on the characteristics in Section 7 in order to screen the closest inbred lines;
- (iii) check of the originality 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.

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.

4.2 *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 For the assessment of uniformity of inbred lines and single hybrids, a population standard of 3% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 40 plants, 3 off-types are allowed. In addition, the same population standard and acceptance probability should apply to clear cases of out-crossed plants in inbred lines as well as plants obviously resulting from the selfing of a parent line in single-cross hybrids (clear difference in plant height, size of ear or earliness as well as proof through isozyme polymorphism).

4.2.3 For three-way cross hybrids, double cross hybrids and open-pollinated varieties, the variability within the variety should not exceed the variability of comparable varieties already known.

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

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 of inbred lines or open-pollinated varieties may be tested, either by growing a further generation, or by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the previous 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) Tassel: time of anthesis (characteristic 8)
- (b) Tassel: anthocyanin coloration at base of glume (characteristic 9)
- (c) Ear: anthocyanin coloration of silks (characteristic 16)
- (d) Plant: length (characteristic 24)
- (e) Ear: type of grain (characteristic 36)
- (f) Excluding varieties with ear type of grain: sweet: Ear: color of dorsal side of grain (characteristic 39)
- (g) Ear: anthocyanin coloration of glumes of cob (characteristic 41)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction.

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*

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.

6.3 *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*

(*) Asterisked characteristic – see Chapter 6.1.2

QL: Qualitative characteristic – see Chapter 6.3

QN: Quantitative characteristic – see Chapter 6.3

PQ: Pseudo-qualitative characteristic – see Chapter 6.3

(S): Possible segregation in three-way and double-cross hybrid varieties

MG, MS, VG: See Chapter 3.3.2

PC: Popcorn variety

SC: Sweet corn variety

(a)-(e) See explanations on the Table of Characteristics in Chapter 8.1

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

14-93 See explanations on the Table of Characteristics in Chapter 8.2 (Decimal Code for the Growth Stages)

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

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
1.	14 (S) VG	First leaf: anthocyanin coloration of sheath	Première feuille: pigmentation anthocyanique de la gaine	Primärblatt: Anthocyanfärbung der Blattscheide	Primera hoja: pigmentación antociánica de la vaina		
QN		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	0674, Jubilee (SC)	1
		weak	faible	gering	débil	MO17, Puma (SC)	3
		medium	moyenne	mittel	media	F252, Gyöngymazsola (SC)	5
		strong	forte	stark	fuerte	F244	7
		very strong	très forte	sehr stark	muy fuerte		9
2.	14 (+) VG	First leaf: shape of apex	Première feuille: forme du sommet	Primärblatt: Form der Spitze	Primera hoja: forma del ápice		
PQ		pointed	pointu	spitz	puntiaguda		1
		pointed to rounded	pointu à arrondi	spitz bis abgerundet	puntiaguda a redondeada	0674	2
		rounded	arrondi	abgerundet	redondeada	Empire (SC), F816	3
		rounded to spatulate	arrondi à spatulé	abgerundet bis spatelförmig	redondeada a espatulada	F259, Merkur (SC)	4
		spatulate	spatulé	spatelförmig	espatulada	EP1	5
3.	51-59 VG	Foliage: intensity of green color	Feuillage: intensité de la couleur verte	Laub: Intensität der Grünfärbung	Follaje: intensidad del color verde		
QN		light	claire	hell	claro	W182E	1
		medium	moyenne	mittel	medio	Empire (SC), W117	2
		dark	foncée	dunkel	oscuro	GSS 3287 (SC), W401	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
4.	51-59 VG	Leaf: undulation of margin of blade	Feuille: ondulation du bord du limbe	Blatt: Wellung des Randes der Spreite	Hoja: ondulación del borde del limbo		
(+)							
QN	(a)	absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	F2	1
		intermediate	moyenne	mittel	media	F252, Puma (SC)	2
		strong	forte	stark	fuerte	Empire (SC), F259	3
5.	65-69 VG	Leaf: angle between blade and stem	Feuille: angle entre le limbe et la tige	Blatt: Winkel zwischen Spreite und Stengel	Hoja: ángulo entre el limbo y el tallo		
(+)							
QN	(a)	very small	très petit	sehr klein	muy pequeño		1
		small	petit	klein	pequeño	A188	3
		medium	moyen	mittel	medio	F66, GH 2547 (SC)	5
		large	grand	groß	grande	F186, Spirit (SC)	7
		very large	très grand	sehr groß	muy grande		9
6.	65-69 VG	Leaf: curvature of blade	Feuille: courbure du limbe	Blatt: Biegung der Spreite	Hoja: curvatura del limbo		
(+)							
QN	(a)	absent or very slightly recurved	absente ou très légèrement incurvé	fehlend oder sehr leicht gebogen	ausente o muy ligeramente recurvada	WD36	1
		slightly recurved	légèrement incurvé	leicht gebogen	ligeramente recurvada	A654, Bonus (SC)	3
		moderately recurved	modérément incurvé	mäßig gebogen	moderadamente recurvada	Jubilee (SC), W117	5
		strongly recurved	fortement incurvé	stark gebogen	fuertemente recurvada	W79A	7
		very strongly recurved	très fortement incurvé	sehr stark gebogen	muy fuertemente recurvada		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
7.	65-69 VG	Stem: degree of zig-zag	Tige: degré du zig-zag	Stengel: Zickzack- ausprägung	Tallo: grado de zigzagueo		
QN		absent or very slight	nul ou très faible	fehlend oder sehr gering	ausente o muy ligero	F2	1
		slight	faible	mäßig	débil	F186	2
		strong	fort	deutlich	fuerte	F66	3
8. (*) (+)	MG	Tassel: time of anthesis	Panicule: époque de floraison mâle	Rispe: Zeitpunkt der männlichen Blüte	Panícula: época de la antesis		
QN	(b)	very early	très précoce	sehr früh	muy temprana		1
		very early to early	très précoce à précoce	sehr früh bis früh	temprana a muy temprana	KW1069, Spirit (SC)	2
		early	précoce	früh	temprana	Champ (SC), F257	3
		early to medium	précoce à moyenne	früh bis mittel	temprana a media	Centurion (SC), F259	4
		medium	moyenne	mittel	media	F522, Zenith (SC)	5
		medium to late	moyenne à tardive	mittel bis spät	media a tardía	A632	6
		late	tardive	spät	tardía	B73	7
		late to very late	tardive à très tardive	spät bis sehr spät	tardía a muy tardía	AM1513	8
		very late	très tardive	sehr spät	muy tardía		9
9. (*) (+)	65-69 (S) VG	Tassel: anthocyanin coloration at base of glume	Panicule: bourrelet (anneau anthocyanique) en-dessous de la glume	Rispe: Anthocyanfärbung an der Basis der Hüllspelze	Panícula: pigmentación antociánica en la base de la gluma		
QN	(b)	absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Royalty (SC), W117	1
		weak	faible	gering	débil	Boston (SC), F66	3
		medium	moyenne	mittel	media	F107	5
		strong	forte	stark	fuerte	EP1	7
		very strong	très forte	sehr stark	muy fuerte		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
10.	65-69	Tassel:	Panicule:	Rispe:	Panícula:		
(+)	(S)	anthocyanin	pigmentation	Anthocyan-	pigmentación		
	VG	coloration of	anthocyanique des	färbung der	antociánica de las		
		glumes excluding	glumes à	Hüllspelze ohne	glumas, con		
		base	l'exclusion de la	Basis	exclusión de la		
		base	base		base		
QN	(b)	absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Empire (SC), F259	1
		weak	faible	gering	débil	F2, Royalty (SC)	3
		medium	moyenne	mittel	media	Centurion (SC), WD36	5
		strong	forte	stark	fuerte	W79A	7
		very strong	très forte	sehr stark	muy fuerte		9
11.	VG	Tassel:	Panicule:	Rispe:	Panícula:		
(+)	(S)	anthocyanin	pigmentation	Anthocyanfärbung	pigmentación		
		coloration of	anthocyanique des	der Antheren	antociánica de las		
		anthers	anthères		antheras		
QN	(b)	absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	A654, Empire (SC)	1
		weak	faible	gering	débil	F2, Royalty (SC)	3
		medium	moyenne	mittel	media	Centurion (SC), W182E	5
		strong	forte	stark	fuerte		7
		very strong	très forte	sehr stark	muy fuerte		9
12.	65-69	Tassel: angle	Panicule: angle	Rispe: Winkel	Panícula: ángulo		
(*)	VG	between main axis	entre l'axe central	zwischen der	entre el eje central		
(+)		and lateral	et les	Mittelachse und den	y las ramas		
		branches	ramifications	Seitenästen	laterales		
		branches	latérales				
QN	(c)	very small	très petit	sehr klein	muy pequeño		1
		small	petit	klein	pequeño	F492	3
		medium	moyen	mittel	medio	EP1, Mv. Aranyos (SC)	5
		large	grand	groß	grande	Bonus (SC), F186	7
		very large	très grand	sehr groß	muy grande		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
13.	69	Tassel: curvature	Panicule:	Rispe: Biegung der	Panícula:		
(*)	(S)	of lateral	courbure des	Seitenäste	curvatura de las		
(+)	VG	branches	ramifications		ramas laterales		
QN	(c)	absent or very slightly recurved	absente ou très légèrement incurvées	fehlend oder sehr leicht gebogen	ausente o muy ligeramente recurvada	El Toro (SC), F257	1
		slightly recurved	légèrement incurvées	leicht gebogen	ligeramente recurvada	Empire (SC), F816	3
		moderately recurved	modérément incurvées	mäßig gebogen	moderadamente recurvada	Bonus (SC), W182E	5
		strongly recurved	fortement incurvées	stark gebogen	fuertemente recurvada	F66	7
		very strongly recurved	très fortement incurvées	sehr stark gebogen	muy fuertemente recurvada		9
14.	65-75	Tassel: number of	Panicule: nombre	Rispe: Anzahl der	Panícula: número		
(*)	MS/	primary lateral	de ramifications	Seitenäste erster	de ramificaciones		
	VG	branches	primaires	Ordnung	primarias		
QN		absent or very few	nul ou très petit	fehlend oder sehr gering	nulo o muy bajo	F7	1
		few	petit	gering	bajo	F252, Mv. Aranyos (SC)	3
		medium	moyen	mittel	medio	F244, Kokanee (SC)	5
		many	grand	groß	grande	A188, Zenith (SC)	7
		very many	très grand	sehr groß	muy grande	Suregold (SC)	9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
15.	MG	Ear: time of silk emergence	Épi: époque d'apparition des soies	Kolben: Zeitpunkt des Erscheinens der Narbenfäden	Mazorca: época de la aparición de los estigmas		
	(+)						
	QN	very early	très précoce	sehr früh	muy temprana	Mv. Aranyos (SC)	1
		very early to early	très précoce à précoce	sehr früh bis früh	temprana a muy temprana	KW1069, Spirit (SC)	2
		early	précoce	früh	temprana	Champ (SC), F257	3
		early to medium	précoce à moyenne	früh bis mittel	temprana a media	F259, Royalty (SC)	4
		medium	moyenne	mittel	media	Bonus (SC), F522	5
		medium to late	moyenne à tardive	mittel bis spät	media a tardía	A632	6
		late	tardive	spät	tardía	B73	7
		late to very late	tardive à très tardive	spät bis sehr spät	tardía a muy tardía	AM1513	8
		very late	très tardive	sehr spät	muy tardía		9
16.	65	Ear: anthocyanin coloration of silks	Épi: pigmentation anthocyanique des soies	Kolben: Anthocyanfärbung der Narbenfäden	Mazorca: pigmentación antocianica de los estigmas		
	(*)						
	(S)						
	VG						
	QN	absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Bonus (SC), F7, F195,	1
		weak	faible	gering	débil	El Toro (SC), F257	3
		medium	moyenne	mittel	media	F244, Gyöngymazsola (SC)	5
		strong	forte	stark	fuerte	W401	7
		very strong	très forte	sehr stark	muy fuerte		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
17.	65-75 (S) VG	Stem: anthocyanin coloration of brace roots	Tige: pigmentation anthocyanique des racines d'ancrage	Stengel: Anthocyanfärbung der Stelzwurzeln	Tallo: pigmentación antociánica de las raíces de anclaje		
QN		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	F16, Jubilee (SC)	1
		weak	faible	gering	débil	Puma (SC), W117	3
		medium	moyenne	mittel	media	El Toro (SC), WD36	5
		strong	forte	stark	fuerte	EP1	7
		very strong	très forte	sehr stark	muy fuerte		9
18.	61-71 VG	Tassel: density of spikelets	Panicule: densité des épillets	Rispe: Dichte der Ährchen	Panícula: densidad de las espiguillas		
QN	(b)	moderately lax	modérément lâche	mäßig locker	moderadamente baja	F16	3
		medium	moyenne	mittel	media	EP1, Royalty (SC)	5
		moderately dense	modérément compacte	mäßig dicht	moderadamente alta	Empire (SC), F259	7
19.	71-75 (S) VG	Leaf: anthocyanin coloration of sheath	Feuille: pigmentation anthocyanique de la gaine	Blatt: Anthocyanfärbung der Blattscheide	Hoja: pigmentación antociánica de la vaina		
QN		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Jubilee (SC), W401	1
		weak	faible	gering	débil	F107	3
		medium	moyenne	mittel	media	F257	5
		strong	forte	stark	fuerte	EP1	7
		very strong	très forte	sehr stark	muy fuerte		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
20.	71-75 (S) VG	Stem: anthocyanin coloration of internodes	Tige: pigmentation anthocyanique des entre-nœuds	Stengel : Anthocyanfärbung der Internodien	Tallo: pigmentación antociánica de los entrenudos		
QN		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	F259, Jubilee (SC)	1
		weak	faible	gering	débil	F816	3
		medium	moyenne	mittel	media	W79A	5
		strong	forte	stark	fuerte	F257	7
		very strong	très forte	sehr stark	muy fuerte		9
21.	71-75 MS	Tassel: length of main axis above <u>lowest</u> lateral branch	Panicule: longueur de l'axe central au-dessus du rameau <u>inférieur</u>	Rispe: Länge der Mittelachse oberhalb des <u>untersten</u> Seitenastes	Panícula: longitud del eje central encima de la rama lateral <u>más baja</u>		
QN		very short	très court	sehr kurz	muy corto		1
		short	court	kurz	corto	EP1	3
		medium	moyen	mittel	medio	Bonus (SC), F244	5
		long	long	lang	largo	Empire (SC), F492	7
		very long	très long	sehr lang	muy largo		9
22.	71-75 (*) MS (+)	Tassel: length of main axis above <u>highest</u> lateral branch	Panicule: longueur de l'axe central au-dessus du rameau <u>supérieur</u>	Rispe: Länge der Mittelachse oberhalb des <u>obersten</u> Seitenastes	Panícula: longitud del eje central encima de la rama lateral <u>más alta</u>		
QN		very short	très court	sehr kurz	muy corto		1
		short	court	kurz	corto	EP1	3
		medium	moyen	mittel	medio	W182E	5
		long	long	lang	largo	F492	7
		very long	très long	sehr lang	muy largo		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
23.	71-75 MS	Tassel: length of lateral branch	Panicule: longueur du rameau	Rispe: Länge der Seitenäste	Panícula: longitud de la rama lateral lateral		
QN	(c)	very short	très court	sehr kurz	muy corta		1
		short	court	kurz	corta	EP1	3
		medium	moyen	mittel	media	A632	5
		long	long	lang	larga	F492	7
		very long	très long	sehr lang	muy larga		9
24.1 (*) (+)	MS 75-85	<u>Only inbred lines and varieties with ear type of grain: sweet or pop:</u> Plant: length	<u>Seulement pour les lignées endogames et les variétés avec type de grain de l'épi: doux ou à éclater:</u> Plante: longueur	<u>Nur Inzuchtlinien und Sorten mit Kolben: Korntyp: Zuckermais oder Popcorn: Pflanze: Länge</u>	<u>Sólo variedades endógamas y variedades con mazorca con tipo de grano: dulce o palomero:</u> Planta: longitud		
QN		very short	très courte	sehr kurz	muy corta	F7	1
		short	courte	kurz	corta	Spirit (SC), W117	3
		medium	moyenne	mittel	media	F244, Puma (SC)	5
		long	longue	lang	larga	Royalty (SC), WD36	7
		very long	très longue	sehr lang	muy larga	Enterprise (SC)	9
24.2 (*) (+)	MS 75-85	<u>Only hybrids and open-pollinated varieties, excluding varieties with ear type of grain: sweet or pop:</u> Plant: length	<u>Seulement pour les hybrides et les variétés à fécondation libre, à l'exclusion des variétés avec type de grain de l'épi: doux ou à éclater:</u> Plante: longueur	<u>Nur Hybriden und freiabblühende Sorten, ohne Sorten mit Kolben: Korntyp: Zuckermais oder Popcorn: Pflanze: Länge</u>	<u>Sólo híbridos y variedades de polinización libre, excepto variedades con mazorca con tipo de grano: dulce o palomero:</u> Planta: longitud		
QN		very short	très courte	sehr kurz	muy corta		1
		short	courte	kurz	corta	PR39D23	3
		medium	moyenne	mittel	media	PR37Y12	5
		long	longue	lang	larga	DKC5166	7
		very long	très longue	sehr lang	muy larga		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
25.	75-85 MG (+)	Plant: ratio height of insertion of peduncle of upper ear to plant length	Plante: hauteur d'insertion du pédoncule de l'épi le plus haut par rapport à la longueur de la plante	Pflanze: Verhältnis der Ansatzhöhe des Kolbenstiels des obersten Kolbens zur Pflanzenlänge	Planta: relación entre la altura de inserción del pedúnculo de la mazorca más alta y la longitud de la planta		
QN		very small	très petit	sehr klein	muy pequeña	Gyöngymazsola (SC)	1
		small	petit	klein	pequeña	F816, Spirit (SC)	3
		medium	moyen	mittel	media	F252, Royalty (SC)	5
		large	grand	groß	grande	F481	7
		very large	très grand	sehr groß	muy grande		9
26.	75-85 MS	Leaf: width of blade	Feuille: largeur du limbe	Blatt: Breite der Spreite	Hoja: anchura del limbo		
QN	(a)	very narrow	très étroit	sehr schmal	muy estrecho		1
		narrow	étroit	schmal	estrecho	Champ (SC), F16	3
		medium	moyen	mittel	medio	Empire (SC), F244	5
		wide	large	breit	ancho	Centurion (SC), F481	7
		very wide	très large	sehr breit	muy ancho		9
27.	75-85 VG	Peduncle: length	Pédoncule: longueur	Kolbenstiel: Länge	Pedúnculo: longitud		
QN		very short	très court	sehr kurz	muy corto		1
		short	court	kurz	corto	Centurion (SC), F259	3
		medium	moyen	mittel	medio	A654, Jubilee (SC)	5
		long	long	lang	largo	F107	7
		very long	très long	sehr lang	muy largo		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
28.	92-93	Ear: length	Épi: longueur	Kolben: Länge	Mazorca: longitud		
(*)	sweet						
(+)	-corn						
	75-79						
	MS						
QN		very short	très court	sehr kurz	muy corta		1
		short	court	kurz	corta	F2	3
		medium	moyen	mittel	media	A654, Spirit (SC)	5
		long	long	lang	larga	Empire (SC), MO17	7
		very long	très long	sehr lang	muy larga		9
29.	92-93	Ear: diameter (in middle)	Épi: diamètre (au milieu)	Kolben: Durchmesser (in der Mitte)	Mazorca: diámetro (en el medio)		
(*)	sweet						
(+)	-corn						
	75-79						
	MS						
QN		very small	très petit	sehr klein	muy pequeño		1
		small	petit	klein	pequeño	F7	3
		medium	moyen	mittel	medio	W117	5
		large	grand	groß	grande	Centurion (SC), F481	7
		very large	très grand	sehr groß	muy grande	Empire (SC)	9
30.	92-93	Ear: shape	Epi: forme	Kolben: Form	Mazorca: forma		
(*)	sweet						
(+)	-corn						
	75-79						
	VG						
QN		conical	conique	konisch	cónica	F16, Wombat (SC)	1
		conico-cylindrical	cylindro-conique	konisch-zylindrisch	cilindrocónica	Centurion (SC), F816	2
		cylindrical	cylindrique	zylindrisch	cilíndrica	F66, GH2547 (SC)	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
31.	92-93 sweet -corn 75-93 MS	Ear: number of rows of grain	Épi: nombre de rangs	Kolben: Anzahl der Kornreihen	Mazorca: número de hileras de granos		
QN		very few	très petit	sehr gering	muy bajo		1
		few	petit	gering	bajo	F257	3
		medium	moyen	mittel	medio	Dessert 73 (SC), F16	5
		many	grand	groß	alto	B73, Bonus (SC)	7
		very many	très grand	sehr groß	muy alto		9
32.	75-79 (S) VG	<u>Only varieties with ear type of grain: sweet or waxy; Ear: number of colors of grains</u>	<u>Seulement pour les variétés avec type de grain de l'épi: doux ou cireux; Épi: nombre de couleurs de grains</u>	<u>Nur Sorten mit Kolben: Korntyp: Zuckermais oder Wachsmals; Kolben: Anzahl Farben der Körner</u>	<u>Sólo variedades con mazorca con tipo de grano: dulce o ceroso; Mazorca: número de colores de los granos</u>		
QL	(e)	one	une	eine	uno	Jubilee (SC)	1
		two	deux	zwei	dos	Eolrukchal-ilho, Serendipity (SC)	2
33. (*)	75-79 VG	<u>Only varieties with ear type of grain: sweet; Grain: intensity of yellow color</u>	<u>Seulement pour les variétés avec le type de grain: doux; Grain: intensité de la couleur jaune</u>	<u>Nur Sorten mit Kolben: Korntyp: Zuckermals; Korn: Intensität der Gelbfärbung</u>	<u>Sólo variedades con mazorca con tipo de grano: dulce; Grano: intensidad del color amarillo</u>		
QN	(e)	light	claire	hell	claro	Gyöngymazsola (SC)	3
		medium	moyenne	mittel	medio	Royalty (SC)	5
		dark	foncée	dunkel	oscuro	Kokanee (SC)	7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
34.	75-79 VG	<u>Only varieties with ear type of grain: sweet:</u> Grain: length	<u>Seulement pour les variétés avec le type de grain de l'épi: doux:</u> Grain: longueur	<u>Nur Sorten mit Kolben: Korntyp:</u> <u>Zuckermals: Korn:</u> Länge	<u>Sólo variedades con mazorca con tipo de grano:</u> <u>dulce: Grano:</u> longitud		
QN	(d)	short	court	kurz	corto		3
		medium	moyen	mittel	medio	Boston (SC)	5
		long	long	lang	largo	GH5704 (SC)	7
35.	75-79 VG (+)	<u>Only varieties with ear type of grain: sweet:</u> Grain: width	<u>Seulement pour les variétés avec le type de grain de l'épi: doux:</u> Grain: largeur	<u>Nur Sorten mit Kolben: Korntyp:</u> <u>Zuckermals: Korn:</u> Breite	<u>Sólo variedades con mazorca con tipo de grano:</u> <u>dulce: Grano:</u> anchura		
QN	(d)	narrow	étroit	schmal	estrecho	Bonus (SC)	3
		medium	moyen	mittel	medio	Jubilee (SC)	5
		broad	large	breit	ancho	Mv. Aranyos (SC)	7
36. (*) (+)	92 (S) VG	Ear: type of grain	Épi: type de grain	Kolben: Korntyp	Mazorca: tipo de grano		
QL	(d)	flint	corné	Hartmais	córneo	F2	1
	(e)	flint-like	corné à corné-denté	hartmaisähnlich	córneo a córneo- dentado	F252	2
		intermediate	corné-denté	Zwischentyp	córneo-dentado	F107	3
		dent-like	corné-denté à denté	zahnmaisähnlich	córneo-dentado a dentado	A654	4
		dent	denté	Zahnmais	dentado	W182E	5
		sweet	sucré	Zuckermals	dulce	Jubilee (SC)	6
		pop	à éclater	Popcorn	palomero	Iowa Pop (PC)	7
		waxy	cireux	Wachsmals	ceroso		8
		flour	farineux	Mehlmais	harinoso		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
37. (*) (+)	92 VG	<u>Only varieties with ear type of grain: sweet:</u> Ear: shrinkage of top of grain	<u>Seulement variétés avec type de grain de l'épi:</u> <u>doux:</u> Épi: rétrécissement de la partie supérieure du grain	<u>Nur Sorten mit Kolben: Korntyp:</u> <u>Zuckermals:</u> Kolben: Schrumpfung Kornkrone	<u>Sólo variedades con mazorca con tipo de grano:</u> <u>dulce:</u> Mazorca: contracción del extremo superior del grano		
QN	(d)	weak	faible	gering	débil	Zarja (SC)	1
	(e)	medium	moyenne	mittel	media	Merkur (SC)	3
		strong	forte	stark	fuerte	Dessert 73 (SC)	5
38. (*)	92-93 (S) VG	Ear: color of top of grain	Épi: couleur du sommet du grain	Kolben: Farbe der Kornkrone	Mazorca: color del extremo superior del grano		
PQ	(d)	white	blanc	weiß	blanco	A188, Pure white (SC), Snowbelle (SC)	1
	(e)	yellowish white	blanc jaunâtre	gelblich weiß	blanco amarillento		2
		yellow	jaune	gelb	amarillo	F259	3
		yellow orange	jaune orangé	gelborange	amarillo anaranjado	F2, Gyöngymazsola (SC)	4
		orange	orange	orange	naranja	F257, GH 2547 (SC)	5
		red orange	rouge orangé	rotorange	naranja rojizo	Dynasty (SC)	6
		red	rouge	rot	rojo		7
		purple	pourpre	purpur	púrpura		8
		brownish	brunâtre	bräunlich	amarronado	Zenith (SC)	9
		blue black	noir-bleu	blauschwarz	negro azulado	Miheukchal	10

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
39. (*)	92-93 (S) VG	<u>Excluding varieties with ear type of grain: sweet: Ear: color of dorsal side of grain</u>	<u>À l'exclusion des variétés avec type de grain de l'épi: doux: Épi: couleur de la face dorsale du grain</u>	<u>Ohne Sorten mit Kolben: Korntyp: Zuckermais: Kolben: Farbe der Kornrückseite</u>	<u>Excepto variedades con mazorca con tipo de grano: dulce: Mazorca: color del lado dorsal del grano</u>		
PQ	(d)	white	blanc	weiß	blanco	F481	1
	(e)	yellowish white	blanc jaunâtre	gelblich weiß	blanco amarillento	A188	2
		yellow	jaune	gelb	amarillo		3
		yellow orange	jaune orangé	gelborange	amarillo anaranjado	F66	4
		orange	orange	orange	naranja	EP1	5
		red orange	rouge orangé	rotorange	naranja rojizo		6
		red	rouge	rot	rojo		7
		purple	pourpre	purpurn	púrpura		8
		brownish	brunâtre	bräunlich	amarronado		9
		blue black	noir-bleu	blauschwarz	negro azulado		10
40. (+)	93 VG	<u>Only varieties with ear type of grain: pop: Type of popped grain</u>	<u>Seulement pour les variétés avec le type de grain de l'épi: à éclater: Type de grain éclaté</u>	<u>Nur Sorten mit Kolben: Korntyp: Popcorn: Typ des gepufften Korns</u>	<u>Sólo variedades con mazorca con tipo de grano: palomero: Tipo del grano reventado</u>		
QN		butterfly	à ailettes	Schmetterlingtyp	palomita	Robust 97461 (PC)	1
		intermediate	intermédiaire	Zwischentyp	intermedio		2
		globular	globuleux	Kugeltyp	globular	Robust 90252 (PC)	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
41.	93 (S)	Ear: anthocyanin	Épi: pigmentation	Kolben: Anthocyan-	Mazorca:		
(*)	VG	coloration of	anthocyanique des	färbung der Spelzen	pigmentación		
(+)		glumes of cob	glumes de la rafle	der Spindel	antociánica de las		
					glumas del zuro		
QN		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	F2, F257	1
		weak	faible	gering	débil	F252	3
		medium	moyenne	mittel	media	W117	5
		strong	forte	stark	fuerte	A632	7
		very strong	très forte	sehr stark	muy fuerte		9

8. Explanations on the Table of Characteristics

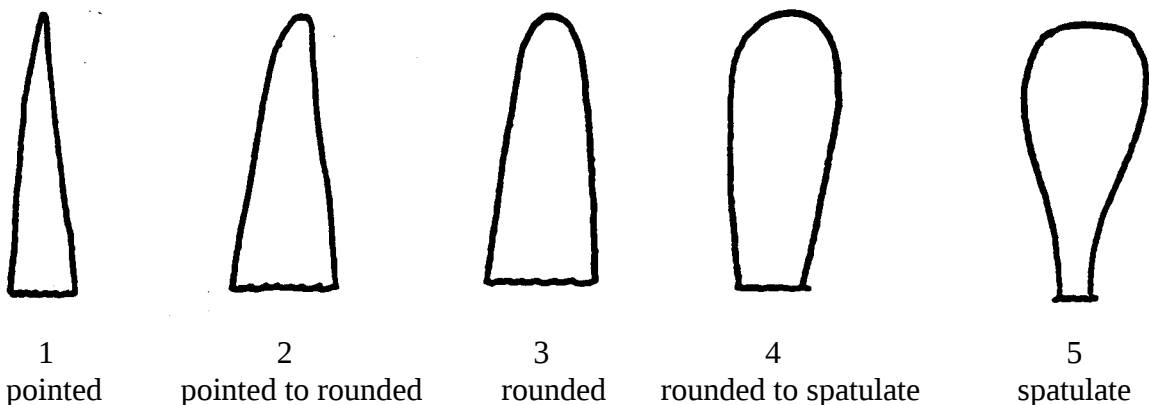
8.1 *Explanations covering several characteristics*

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

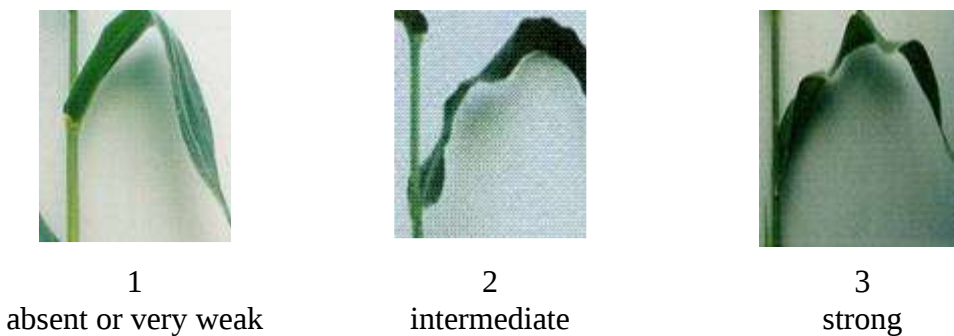
- (a) The observation should be made on the leaf just above upper ear.
- (b) The observation should be made in the middle third of the main branch of the tassel.
- (c) The observation should be made on the second branch from the bottom of the tassel.
- (d) The observation should be made in the middle third of the uppermost ear, when well developed.
- (e) This characteristic may be influenced by cross-pollination. In particular in sweetcorn and popcorn varieties, cross-pollination should be avoided.

8.2 *Explanations for individual characteristics*

Ad. 2: First leaf: shape of apex

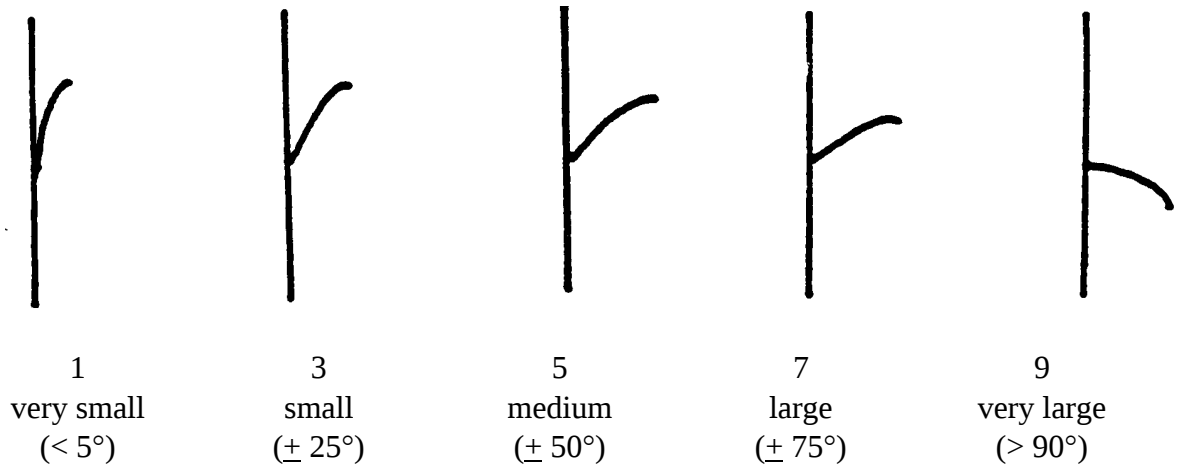


Ad. 4: Leaf: undulation of margin of blade



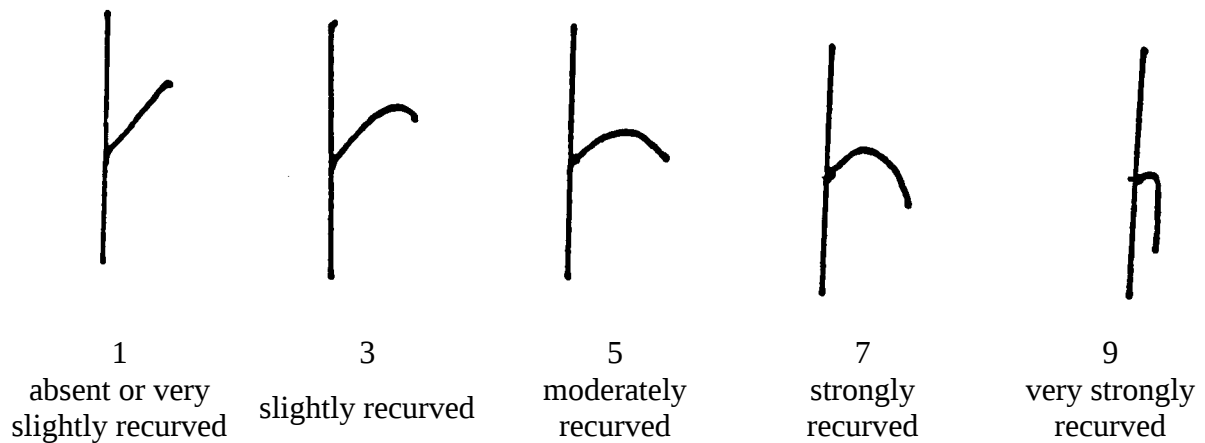
Ad. 5: Leaf: angle between blade and stem

Ad. 12: Tassel: angle between main axis and lateral branches



Ad. 6: Leaf: curvature of blade

Ad. 13: Tassel: curvature of lateral branches

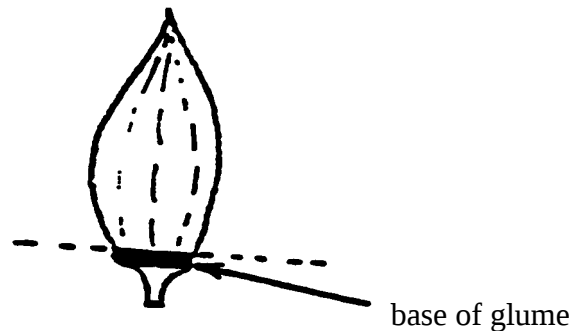


Ad. 8: Tassel: time of anthesis

The time of anthesis is when 50% of plants have anthers visible in the middle third of the main branch.

Ad. 9: Tassel: anthocyanin coloration at base of glume

Ad. 10: Tassel: anthocyanin coloration of glumes excluding base



Ad. 11: Tassel: anthocyanin coloration of anthers

The observation should be made in the middle third of the main branch on fresh anthers.

Ad. 15: Ear: time of silk emergence

The time of silk emergence is when silks have emerged on 50% of plants.

Ad. 17: Stem: anthocyanin coloration of brace roots

The observation should be made when well developed and fresh brace roots are present on 50% of plants.

Ad. 19: Leaf: anthocyanin coloration of sheath

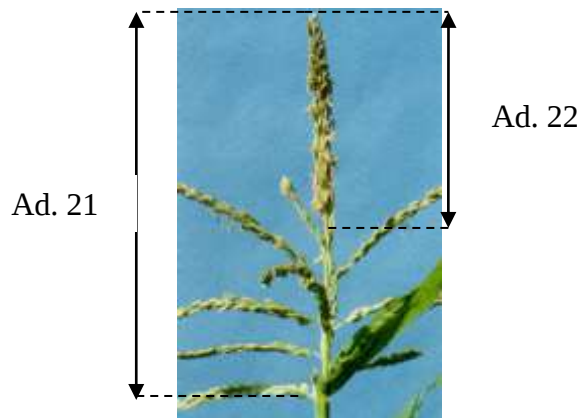
The observation should be made in the middle third of the plant.

Ad. 20: Stem: anthocyanin coloration of internodes

The observation should be made just above insertion point of peduncle of upper ear.

Ad. 21: Tassel: length of main axis above lowest lateral branch

Ad. 22: Tassel: length of main axis above highest lateral branch



Ad. 24.1: Only inbred lines and varieties with ear type of grain: sweet or pop: Plant: length

Ad. 24.2: Only hybrids and open-pollinated varieties, excluding varieties with ear type of grain: sweet or pop: Plant: length

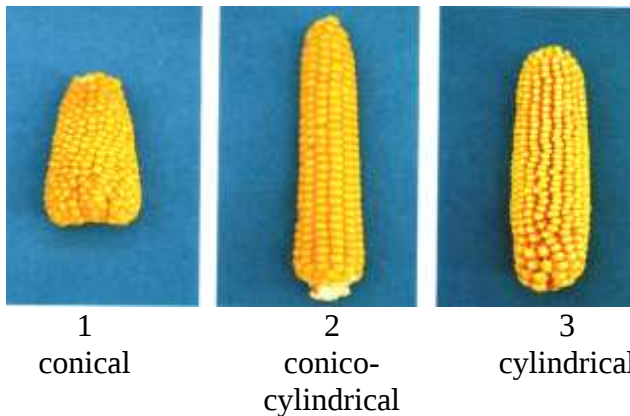
Ad. 25: Plant: ratio height of insertion of peduncle of upper ear to plant length

The plant length should be observed including the tassel.

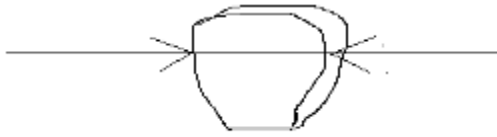
Ad. 28: Ear: length



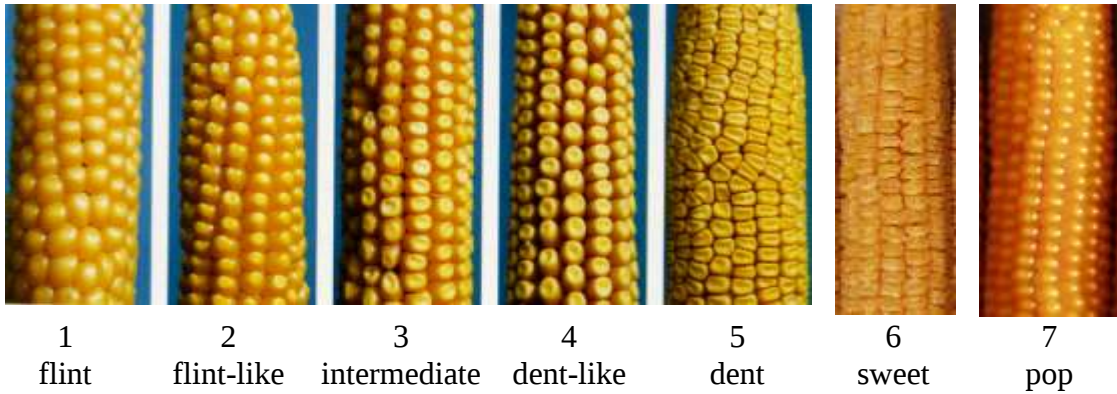
Ad. 30: Ear: shape



Ad. 35: Only varieties with ear type of grain: sweet: Grain: width



Ad. 36: Ear: type of grain



1	flint	mostly hard endosperm, round grain, thick layer of hard endosperm on crown, larger grains than pop
2	flint-like	mostly hard endosperm, round grain, intermediate layer of hard endosperm on crown
3	intermediate	thin layer of hard endosperm on crown, crown slightly indented
4	dent-like	mostly soft endosperm, crown moderately indented, medium layer of hard endosperm on dorsal side of grain,
5	dent	mostly soft endosperm covering also exterior part of crown, thin layer of hard endosperm only on dorsal side of grain, grain strongly indented on crown
6	sweet	glassy endosperm with very low or no starch content, wrinkled grain
7	pop	nearly completely hard endosperm, rice-type (pointed grain) or pearl type (rounded grain), very thick layer of hard endosperm on crown, smaller grains than flint
8	waxy	approximately 100 % amylopectine, waxy appearance of grain, pink coloration of endosperm in iodine staining test (blue black coloration of other types of grain). <u>Iodine staining test</u>   waxy non waxy
9	flour	completely soft endosperm, grain round or slightly indented on crown

Ad. 37: Only varieties with ear type of grain: sweet: Ear: shrinkage of top of grain



1
weak



3
medium



5
strong

Ad. 40: Only varieties with ear type of grain: pop: Type of popped grain

Ears should be stored for a minimum of 2 or 3 months after harvest before popping.

The dry grains (13-13.5% water content is optimal) are popped with heating. The typical shape of the popped grains has to be observed.



1
butterfly



3
globular

Ad. 41: Ear: anthocyanin coloration of glumes of cob

The anthocyanin coloration should be observed on the middle third of the uppermost cob, after the removal of some of the grains.

8.3 Decimal Code for the Growth Stages*

This decimal code is in close conformity with the BBCH-code (Meier, 1997)

CODE	GENERAL DESCRIPTION		DESCRIPTION
	<u>Seedling growth</u>	<u>Croissance de la plantule</u>	<u>Wachstum des Keimlings</u>
14	4 leaves unfolded	4 feuilles étalées	4 Blätter entfaltet
	<u>Tillering</u>	<u>Tallage</u>	<u>Bestockung</u>
	<u>Stem elongation</u>	<u>Elongation de la tige</u> (montaison)	<u>Schossen</u>
	<u>Booting</u>	<u>Gonflement</u>	<u>Schwellstadium</u>
	<u>Inflorescence emergence</u>	<u>Epiaison</u>	<u>Erscheinen des</u> <u>Blütenstands</u>
51 (♂,♀)	Inflorescence just visible	Inflorescence à peine visible	Blütenstand gerade sichtbar
59	Emergence of inflorescence completed	Inflorescence complètement dégagée	Blütenstand vollständig geschoben
(♂,♀)			
	<u>Anthesis</u>	<u>Anthèse</u>	<u>Blüte</u>
61	Beginning of anthesis	Début de l'anthèse	Beginn der Blüte
65	Anthesis halfway	Mi-floraison	Mitte der Blüte
69	Anthesis complete	Anthèse complete	Ende der Blüte
	<u>Milk development</u>	<u>Stade laiteux</u>	<u>Entwicklung der Milchreife</u>
71	Caryopsis watery ripe	State aqueux de la maturation du caryopse	Karyopse wasserreif
73	Early milk	début laiteux	
75	Medium milk	Mi-laiteux	Mitte der Milchreife

79(1)	Grains have reached final size	Le grain a atteint la taille finale	Körner haben Endgröße erreicht
85	<u>Dough development</u> Soft dough	<u>Stade pâteux</u> Pâteux tendre	<u>Entwicklung der Teigreife</u> weich teigreif
92	<u>Ripening</u> Caryopsis hard (can no longer be dented by thumbnail)	<u>Maturation</u> Le caryopse est dur (ne peut plus du tout être entamé par l'ongle)	<u>Das Reifen</u> Karyopse hart (nicht mehr mit dem Daumennagel einzudellen)
93	Caryopsis loosening in daytime	Caryopse se détachant dans la journée	Karyopse tagsüber lockernd

-
- * Extracted from J.C. Zadoks, T.T. Chang and C.F. Konzak except (1), Decimal Code for the Growth States of Cereals, EUCARPIA Bulletin No. 7, 1974, pp. 42-52. The French translation has been kindly furnished by Mrs. R. Cassini, Mr. R. Cassini and Mr. R. Marie. The German translation has been kindly furnished by Mr. A.O. Klomp and Mrs. I. Volk.
- * Extrait de J.C. Zadoks, T.T. Chang et C.F. Konzak excepté (1), Decimal Code for the Growth States of Cereals, EUCARPIA Bulletin No. 7, 1974, pp. 42-52. La traduction française a été aimablement fournie par Mme R. Cassini, M. R. Cassini et M. R. Marie. La traduction allemande a été aimablement fournie par M. A.O. Klomp et Mme I. Volk.
- * Auszug von J.C. Zadoks, T.T. Chang und C.F. Konzak außer (1), Decimal Code for the Growth States of Cereals, EUCARPIA Bulletin No. 7, 1974, pp. 42-52. Die französische Übersetzung wurde freundlicherweise von Frau R. Cassini, Herrn R. Cassini und Herrn R. Marie überlassen. Die deutsche Uebersetzung wurde freundlicherweise von Herrn A.O. Klomp und Frau I. Volk überlassen.
- * Extraído de J.C. Zadoks, T.T. Chang y C.F. Konzak excepto (1), Decimal Code for the Growth States of Cereals, EUCARPIA Bulletin No. 7, 1974, pp. 42-52. La traducción al francés ha sido facilitada amablemente por la Sra. R. Cassini, el Sr. R. Cassini y el Sr. R. Marie. La traducción al alemán ha sido facilitada amablemente por el Sr. A.O. Klomp y la Sra. I. Volk.

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10. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
		Application date: (not to be filled in by the applicant)
TECHNICAL QUESTIONNAIRE to be completed in connection with an application for plant breeders' rights		
1. Subject of the Technical Questionnaire		
1.1 Botanical name	Zea mays L.	
1.2 Common name	Maize	
2. Applicant		
Name		
Address		
Telephone No.		
Fax No.		
E-mail address		
Breeder (if different from applicant)		
3. Proposed denomination and breeder's reference		
Proposed denomination (if available)		
Breeder's reference		

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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#4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

(i)	Inbred line	[]
(ii)	Single-cross hybrid	[]
(iii)	Three-way cross hybrid	[]
(iv)	Double-cross hybrid	[]
(v)	Open-pollinated variety	[]
(vi)	Other (provide details)	[]

Variety resulting from:

4.1.1 Crossing

(a)	controlled cross (please state parent varieties)	[]
(b)	partially known cross (please state known parent variety(ies))	[]
(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.

Authorities may choose to request this information

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
4.2.2 Open-pollinated variety (please provide details) 4.2.3 Other (please provide details) 		

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 Tassel: time of anthesis (8)		
very early		1 []
very early to early	KW1069, Spirit (SC)	2 []
early	Champ (SC), F257	3 []
early to medium	Centurion (SC), F259	4 []
medium	F522, Zenith (SC)	5 []
medium to late	A632	6 []
late	B73	7 []
late to very late	AM1513	8 []
very late		9 []
5.2 Tassel: anthocyanin coloration at base of glume (9)		
absent or very weak	Royalty (SC), W117	1[]
very weak to weak		2[]
weak	Boston (SC), F66	3[]
weak to medium		4[]
medium	F107	5[]
medium to strong		6[]
strong	EP1	7[]
strong to very strong		8[]
very strong		9[]

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
Characteristics	Example Varieties	Note	
5.3 Tassel: anthocyanin coloration of glumes excluding base (10)			
absent or very weak	Empire (SC), F259	1[]	
very weak to weak		2[]	
weak	F2, Royalty (SC)	3[]	
weak to medium		4[]	
medium	Centurion (SC), WD36	5[]	
medium to strong		6[]	
strong	W79A	7[]	
strong to very strong		8[]	
very strong		9[]	
5.4 Tassel: anthocyanin coloration of anthers (11)			
absent or very weak	A654, Empire (SC)	1[]	
very weak to weak		2[]	
weak	F2, Royalty (SC)	3[]	
weak to medium		4[]	
medium	Centurion (SC), W182E	5[]	
medium to strong		6[]	
strong		7[]	
strong to very strong		8[]	
very strong		9[]	

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
Characteristics	Example Varieties	Note	
5.5 Tassel: number of primary lateral branches (14)			
absent or very few	F7	1[]	
very few to few		2[]	
few	F252, Mv. Aranyos (SC)	3[]	
few to medium		4[]	
medium	F244, Kokanee (SC)	5[]	
medium to many		6[]	
many	A188, Zenith (SC)	7[]	
many to very many		8[]	
very many	Suregold (SC)	9[]	
5.6 Ear: time of silk emergence (15)			
very early	Mv. Aranyos (SC)	1[]	
very early to early	KW1069, Spirit (SC)	2[]	
early	Champ (SC), F257	3[]	
early to medium	F259, Royalty (SC)	4[]	
medium	Bonus (SC), F522	5[]	
medium to late	A632	6[]	
late	B73	7[]	
late to very late	AM1513	8[]	
very late		9[]	

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
Characteristics	Example Varieties	Note	
5.7 Ear: anthocyanin coloration of silks (16)			
absent or very weak	Bonus (SC), F7, F195	1[]	
very weak to weak		2[]	
weak	El Toro (SC), F257	3[]	
weak to medium		4[]	
medium	F244, Gyöngymazsola (SC)	5[]	
medium to strong		6[]	
strong	W401	7[]	
strong to very strong		8[]	
very strong		9[]	
5.8 i Only inbred lines and varieties with ear type of grain: sweet or pop: (24.1) Plant: length			
very short	F7	1[]	
very short to short		2[]	
short	Spirit (SC), W117	3[]	
short to medium		4[]	
medium	F244, Puma (SC)	5[]	
medium to long		6[]	
long	Royalty (SC), WD36	7[]	
long to very long		8[]	
very long	Enterprise (SC)	9[]	

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.4 8 ii <u>Only hybrids and open-pollinated varieties, excluding varieties with ear</u> (24.2) <u>type of grain: sweet or pop: Plant: length</u>		
very short		1[]
very short to short		2[]
short	PR39D23	3[]
short to medium		4[]
medium	PR37Y12	5[]
medium to long		6[]
long	DKC5166	7[]
long to very long		8[]
very long		9[]
5.9 Ear: length (28)		
very short		1[]
very short to short		2[]
short	F2	3[]
short to medium		4[]
medium	A654, Spirit (SC)	5[]
medium to long		6[]
long	Empire (SC), MO17	7[]
long to very long		8[]
very long		9[]

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
Characteristics	Example Varieties	Note	
5.10 Ear: diameter (in middle) (29)			
very small		1[]	
very small to small		2[]	
small	F7	3[]	
small to medium		4[]	
medium	W117	5[]	
medium to large		6[]	
large	Centurion (SC), F481	7[]	
large to very large		8[]	
very large	Empire (SC)	9[]	
5.11 Ear: shape (30)			
conical	F16, Wombat (SC)	1[]	
conico-cylindrical	Centurion (SC), F816	2[]	
cylindrical	F66, GH2547 (SC)	3[]	
5.12 Ear: number of rows of grain (31)			
very few		1[]	
very few to few		2[]	
few	F257	3[]	
few to medium		4[]	
medium	Dessert 73 (SC), F16	5[]	
medium to many		6[]	
many	B73, Bonus (SC)	7[]	
many to very many		8[]	
very many		9[]	
5.13 <u>Only varieties with ear type of grain: sweet or waxy:</u> Ear: number of colors of grains (32)			
one	Jubilee (SC)	1[]	
two	Eolrukchal-ilho, Serendipity (SC)	2[]	

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.14 (33) <u>Only varieties with ear type of grain: sweet: Grain: intensity of yellow color</u>		
very light		1[]
very light to light		2[]
light	Gyöngymazsola (SC)	3[]
light to medium		4[]
medium	Royalty (SC)	5[]
medium to dark		6[]
dark	Kokanee (SC)	7[]
dark to very dark		8[]
very dark		9[]
5.15 (35) <u>Only varieties with ear type of grain: sweet: Grain: width</u>		
very narrow		1[]
very narrow to narrow		2[]
narrow	Bonus (SC)	3[]
narrow to medium		4[]
medium	Jubilee (SC)	5[]
medium to broad		6[]
broad	Mv. Aranyos (SC)	7[]
broad to very broad		8[]
very broad		9[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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	Characteristics	Example Varieties	Note
5.16 (36)	Ear: type of grain		
	flint	F2	1 []
	flint-like	F252	2 []
	intermediate	F107	3 []
	dent-like	A654	4 []
	dent	W182E	5 []
	sweet	Jubilee (SC)	6 []
	pop	Iowa Pop (PC)	7 []
	waxy		8 []
	flour		9 []
5.17 (37)	<u>Only varieties with ear type of grain: sweet:</u> Ear: shrinkage of top of grain		
	weak	Zarja (SC)	1[]
	weak to medium		2[]
	medium	Merkur (SC)	3[]
	medium to strong		4[]
	strong	Dessert 73 (SC)	5[]

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.18 (38) Ear: color of top of grain		
white	A188, Pure white (SC), Snowbelle (SC)	1 []
yellowish white		2 []
yellow	F259	3 []
yellow orange	F2, Gyöngymazsola (SC)	4 []
orange	F257, GH 2547 (SC)	5 []
red orange	Dynasty (SC)	6 []
red		7 []
purple		8 []
brownish	Zenith (SC)	9 []
blue black	Miheukchal	10 []
5.19 (39) <u>Excluding varieties with ear type of grain: sweet:</u> Ear: color of dorsal side of grain		
white	F481	1 []
yellowish white	A188	2 []
yellow		3 []
yellow orange	F66	4 []
orange	EP1	5 []
red orange		6 []
red		7 []
purple		8 []
brownish		9 []
blue black		10 []

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
Characteristics		Example Varieties	Note
5.20	Ear: anthocyanin coloration of glumes of cob		
(41)			
	absent or very weak	F2, F257	1 []
	very weak to weak		2 []
	weak	F252	3 []
	weak to medium		4 []
	medium	W117	5 []
	medium to strong		6 []
	strong	A632	7 []
	strong to very strong		8 []
	very strong		9 []

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>Ear: time of silk emergence</i>	<i>early</i>	<i>early to medium</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

Sweetcorn varieties only: type

normal sweet (su1)	Jubilee (SC)	1 ☐
sugary enhanced (se)	Gyöngymazsola (SC)	2 ☐
super sweet (sh2)	Zenith (SC)	3 ☐
other (please specify)		4 ☐

Other information

Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

ANNEX

Additional Useful Explanations

<u>TABLE OF CONTENTS</u>	<u>PAGE</u>
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Part II. Characteristics derived by Isozyme Polymorphism	46
Part III. Description of the SGE Method for the Analysis of Isozymes from <i>Zea mays</i> L.	50

Part I

Introduction

The following Annex contains a list of characteristics based on isozyme markers revealed by 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 based on isozyme markers revealed by 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.

For the analysis of isozymes, starch gel electrophoresis is recommended. Polymorphism of isozymes (i.e. 16 enzyme loci) can be detected. Genetic control is known for each enzyme locus. For the description of the method and the genetic interpretation of the zymograms, reference is made to the technical bulletin by Stuber, Wendel, Goodman and Smith, 1988, and the technical handbook by Grenèche and Giraud, 1994. The alleles are described by band numbers according to the definition given by Cardy, Stuber, Goodman, 1980, (see Chapter IX, Literature).

Part II

Characteristics derived by Isozyme Polymorphism

Characteristics		Examples		Note
42. QL	<i>Allele expression at locus Mdh 1</i>	Genotype 1/1	F252	1
		Genotype 0.5/0.5	R3126	
		Genotype 0.5/1	KW 5361 xKW 5454	
		Genotype 1/6 in interaction with allele 6 of Mdh 2	Tau	
		Genotype 0.5/6 in interaction with allele 6 of Mdh 2	Clarica	2
		Genotype 6/6	A239	
		Genotype 1/6 but not in interaction with allele 6 of Mdh 2	Marshall	
		Genotype 0.5/6 but not in interaction with allele 6 of Mdh 2	DK231	

Characteristics		Examples		Note
43. QL	<i>Allele expression at locus Mdh 2</i>	Genotype 3/3	F252	1
		Genotype 3.5/3.5	R3126	
		Genotype 3/3.5	Limit, DK 231	
		Genotype 3/4.5	Robin	
		Genotype 3.5/4.5		
		Genotype 4.5/4.5	W401	2
		Genotype 6/6	A239	3
		Genotype 3/6	Azur	4
		Genotype 3.5/6	Clarica	
		Genotype 4.5/6		5
44. QL	<i>Allele expression at locus Mdh 3</i>	Genotype 16/16	F252	1
		Genotype 18/18	CO 158	2
		Genotype 16/18	Figaro	3
45. QL	<i>Allele expression at locus Mmm</i>	Genotype M/M	F252	1
		Genotype M/m		
		Genotype m/m	86 N 42	2
46. QL	<i>Allele expression at loci Mdh 4 + Mdh 5</i>	Genotype 12/12 + 12/12	F252	1
		Genotype 12/12 + 15/15	F 2	2
		Genotype 12/12 + 12/15	Robin	
47. QL	<i>Allele expression at loci Idh1 + Idh 2</i>	Genotype 4/4 + 4/4	A239	1
		Genotype 4/6 + 4/4		
		Genotype 4/4 + 6/6	CM7	2
		Genotype 6/6 + 4/4	F1110	3
		Genotype 6/6 + 6/6	CO 158	4
		Genotype 4/6 + 6/6	Bonny	
		Genotype 4/4 + 4/6	Axon	5
		Genotype 4/6 + 4/6	Loft	
		Genotyp 6/6 + 4/6		6

Characteristics		Examples	Note	
48. QL	<i>Allele expression at loci Pgd 1 + Pgd2</i>	Genotype 2/2 + 5/5	W401	1
		Genotype 2/2 + 2.8/2.8 Genotype 2/2 + n/n	SK 203	2
		Genotype 3.8/3.8 + 2.8/2.8 Genotype 3.8/3.8 +n/n	A632	3
		Genotype 3.8/3.8 + 5/5 Genotype 3.8/3.8 + 2.8/5 Genotype n/3.8 + 5/5	F252 Tekila	4
		Genotype n/n + 5/5	H108	5
		Genotype 2/3.8 + 5/5 Genotype 2/3.8 + 2.8/5	Bekefix Furio	6
		Genotype 2/2 + 2.8/5	NX 6032	7
	49. PQ	<i>Allele expression at loci Pgm 1 + Pgm2</i>	Genotype 9/9 + 1/1	F 2
		Genotype 9/9 + 1/3	Robin	2
		Genotype 9/9 + 3/3	F 16	3
		Genotype 9/9 + 3/4	Figaro	4
		Genotype 9/9 + 4/4	A 632	5
		Genotype 9/9 + 1/4	Axon	6
		Genotype 9/9 + 8/8	MO 17	7
		Genotype 9/9 + 3/8		8
		Genotype 9/9 + 4/8	Occitan	9
		Genotype 9/9 + 1/8		10
		Genotype 16/16 + 1/1		11
		Genotype 16/16 + 1/3		12
		Genotype 16/16 + 3/3	9034	13
		Genotype 16/16 + 4/4		14
		Genotype 16/16 + 8/8	F 492	15
		Genotype 5/5+3/3	D 06	16
50. QL	<i>Allele expression at locus Pgi 1</i>	Genotype 4/4	A239	1
		Genotype 5/5	A632	2
		Genotype 4/5	Artist	3

Characteristics		Examples	Note	
51. PQ	<i>Allele expression at locus Acp1</i>	Genotype 2/2	F 2	1
		Genotype 2/3	Azur	2
		Genotype 3/3	A 239	3
		Genotype 4/6	Contessa	4
		Genotype 4/4	A 632	5
		Genotype 6/6	F 1444	6
		Genotype 2/4	Occitan	7
		Genotype 2/6		8
		Genotype 3/4	Marshall	9
		Genotype 3/6		10
52. QL	<i>Allele expression at locus Dia 1</i>	Genotype 8/8	F 2	1
		Genotype 12/12	CO 158	2
		Genotype 8/12	Bastion	3
53. QL	<i>Allele expression at locus Dia2</i>	Genotype 4/4	F 2	1
		Genotype 6/6	34 M838	2
		Genotype 4/6	31 N 6	3
54. QL	<i>Allele expression at locus Adh 1</i>	Genotype 4/4	F 1444	1
		Genotype 6/6	F 2	2
		Genotype 4/6	Bristol	3

Part III

Description of the SGE Method for the Analysis of Isozymes from *Zea mays* L.

1. Number of coleoptiles per test

- for checking formula: at least 20 coleoptiles of each inbred line
2 coleoptiles of single-cross hybrids
6 coleoptiles of three-way cross hybrids
- for distinctness, uniformity and stability test: at least 20 coleoptiles 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

L-Ascorbic acid
L-Ascorbic acid Na salt
Sucrose

3.2 Chemicals for electrophoresis

Bromophenol blue
Citric acid monohydrate
L-Histidine
Starch hydrolyzed, for electrophoresis,)

3.3 Chemicals for staining enzymes

Acetic acid glacial
2,6-Dichlorophenol-indophenol Na salt
Ethanol
Ethylenediamine tetra-acetic acid Na₂ Salt (EDTA)
Fast Garnet GBC salt
D-Fructose 6-phosphate Na₂ salt
Glucose 1-phosphate dehydrogenase (Serva 22820 or 22822 or Sigma G5885)
Hydrochloric acid (HCl)
DL-Isocitric acid Na₃ salt
Magnesium chloride hexahydrate
DL-Malic acid
Dimethylthiazol diphenyl tetrazolium (MTT)
β -Nicotinamide adenine dinucleotide (NAD)

β -Nicotinamide adenine dinucleotide reduced (NADH)
 β -Nicotinamide adenine dinucleotide phosphate (NADP)
Nitro-blue tetrazolium (NBT)
Sodium hydroxide (NaOH)
1-Naphtyl acid phosphate
6-phosphogluconic acid Na₃ salt dihydrate
Phenazine methosulfate (PMS)
Polyvinylpyrrolidone 40 (PVP-40)
Sodium acetate trihydrate
Tris-(hydroxymethyl) aminomethane (Tris)

4. Solutions

4.1 Extraction solution

16.7 g Sucrose
8.3 g sodium ascorbate
made up to 100 ml with de-ionised water and adjusted to pH 7.4 with L-ascorbic acid.

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
8.20 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 5.0

4.2.2.1 Running buffer: 0.074 M L-histidine-citrate pH 5.0
15.5g L-histidine
10.0g Citric acid monohydrate
made up to 2 liters with de-ionised water

4.2.2.2 Gel buffer: 0.006 M L-histidine-citrate
(Running buffer diluted 1 in 12)
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 8.0
121.1g Tris, made up to 1 liter with de-ionised water and adjusted to pH 8.0 with 50% HCl
- 4.3.1.2 1 M Tris-HCl pH 9.1
121.1 g Tris, made up to 1 liter with de-ionised water and adjusted to pH 9.1 with 50% HCl
- 4.3.1.3 1 M Sodium acetate pH 5.0
136.08 g Sodium acetate trihydrate, made up to 1 liter with de-ionised water adjusted to pH 5.0 with acetic acid glacial
- 4.3.1.4 MTT solution
1.0 g MTT made up to 100 ml with de-ionised water
- 4.3.1.5 NBT solution
1.0 g NBT made up to 100 ml with de-ionised water
- 4.3.1.6 PMS solution
200 mg PMS, made up to 100 ml with de-ionised water
- 4.3.1.7 MgCl₂ solution
21.35 g Magnesium chloride hexahydrate
made up to 100 ml with de-ionised water
- 4.3.1.8 Malic acid solution
5 g LL-Malic acid, made up to 100 ml with de-ionised water and adjusted to pH 8.0 with 1 M NaOH

4.3.2 Staining solutions (volume: 200 ml)

- 4.3.2.1 MDH + ADH staining solution
20 ml Tris-HCl pH 9.1 (4.3.1.2.)
+ 180 ml de-ionised water
+ 8 ml Malic acid solution (4.3.1.8.)
+ 10 ml Ethanol
+ 80 mg NAD
+ 4 ml NBT solution (4.3.1.5.)
+ 3 ml PMS solution (4.3.1.6.)
- 4.3.2.2 IDH staining solution
20 ml Tris-HCl pH 8.0 (4.3.1.5.)
+ 180 ml de-ionised water
+ 500 mg DL-Isocitric acid Na₃ salt
+ 10 ml MgCl₂ solution (4.3.1.7.)
+ 6 mg NADP
+ 4 ml MTT solution (4.3.1.4.)
+ 3 ml PMS solution (4.3.1.6.)
- 4.3.2.3 PGI + PGD staining solution
20 ml Tris-HCl pH 8.0 (4.3.1.1.)
+ 180 ml de-ionised water
+ 200 mg Fructose 6-phosphate Na₂ salt
+ 80 mg 6-Phosphogluconic acid Na₃ salt trihydrate
+ 2 ml MgCl₂ solution (4.3.1.7.)
+ 20 mg NADP

- + 2 ml MTT solution (4.3.1.4.)
- + 3 ml PMS solution (4.3.1.6.)
- + 50 units Glucose 6-phosphate dehydrogenase

- 4.3.2.4 PGM staining solution
20 ml Tris-HCl pH 8.0 (4.3.1.1.)
+ 180 ml de-ionised water
+ 1 g Glucose 1-phosphate
+ 200 mg EDTA Na₂ salt
+ 4 ml MgCl₂ solution (4.3.1.7.)
+ 20 mg NADP
+ 3 ml MTT solution (4.3.1.4.)
+ 2 ml PMS solution (4.3.1.6.)
+ 100 units Glucose 6-phosphate dehydrogenase
- 4.3.2.5 ACP staining solution
4 ml Sodium acetate pH 5.0 (4.3.1.3.)
+ 196 ml de-ionised water
+ 200 mg Fast Garnet GBC salt
+ 492 mg 1-Naphthylphosphate Na₃ salt dihydrate
+ 2 ml MgCl₂ solution (4.3.1.7.)
- 4.3.2.6 DIA staining solution
20 ml Tris-HCl pH 9.1 (4.3.1.2.)
+ 180 ml de-ionised water
+ 2 g PVP-40
+ 20 mg NADH
+ 16 ml MTT solution (4.3.1.4.)
+ 16 mg 2,6-Dichlorophenol-indophenol Na salt

5. Procedure

5.1 Enzyme extraction

Maize seedlings are grown on moistened germination paper or in a box with sand or vermiculite, at 25°C, in darkness. After five days, individual coleoptiles are cut at 15 mm from the tip and homogenized at 4°C, with a pestle in micro-tubes containing 0.060 ml extraction solution (3.1). The tubes are then centrifuged at 4°C to obtain a clear supernatant. The extracts can be stored at - 30°C.

5.2 Preparation of the gel

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 Buchner flask at 80°C. The mixture is degassed for 40 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 at least two hours, and wrapped with polyethylene film for overnight storage. Before electrophoresis, the gels are cooled at 4°C for at least one hour.

5.3 Electrophoresis

5.3.1 The tanks are filled with the appropriate volume of running buffer (4.2.1.2. or 4.2.2.1.) pre-cooled to 4°C. A slit is cut in the gel at 1 cm from the cathode. The enzyme extracts from 5.1 (30 extracts for on 18 x 18 x 1 cm gel) are absorbed onto 15 x 2 x 1 mm wicks at from Whatman N° 3 chromatography paper. The wicks are placed into the slit. At 1 cm of each edge of the gels, a wick soaked with bromophenol blue solution (4.2.2.3.) is inserted. The electrophoresis is carried out at 4°C. A constant voltage of 200 V (maximum current of 150 mA for two 18 x 18 x 1 cm gels is applied for 20 minutes). The wicks are then removed and the electrophoresis is continued at a constant voltage of 280 V (maximum current of 180 mA for two 18 x 18 x 1 cm gels), until the bromophenol blue marker has migrated 14 cm (4 hours).

5.4 Enzyme staining

After electrophoresis the gel is cut horizontally in 1 mm thick slices. The upper slice is discarded. Individual gel slices are stained by incubation in the following solutions at 37°C in darkness.

for MDH and ADH:	solution 4.3.2.1.,	for IDH:	solution 4.3.2.2.
for PGI and PGD:	solution 4.3.2.3.,	for PGM:	solution 4.3.2.4.
for ACP:	solution 4.3.2.5.,	for DIA:	solution 4.3.2.6

The ACPs migrate in the first 4 cm of the gel; the PGMs go further; therefore, it is possible to stain these two enzymes on the same gel after having cut it transversally.

The staining times range between 30 and 120 minutes. After staining the gel slices are rinsed in distilled water before being stored. The following procedure for long time storing can be successfully used: e.g. drying the gels between two cellophane sheets or storing in sealed polythene bags.

6. Recognition of the alleles encoding isozymes

6.1 Recognition of the alleles encoding MDH

6.1.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal location	Locus	Alleles*	
		8	Mdh1	0,5; 1; 6; 10,5; n	
		6L	Mdh2	3; 3,5; 4,5; 6; n	intergenic
Malate dehydrogenase (MDH)	Dimeric	3L	Mdh3	16; 18	interactions
		1L	Mmm	M; m	
		1L	Mdh4	12	intergenic
		5S	Mdh5	12; 15	interactions

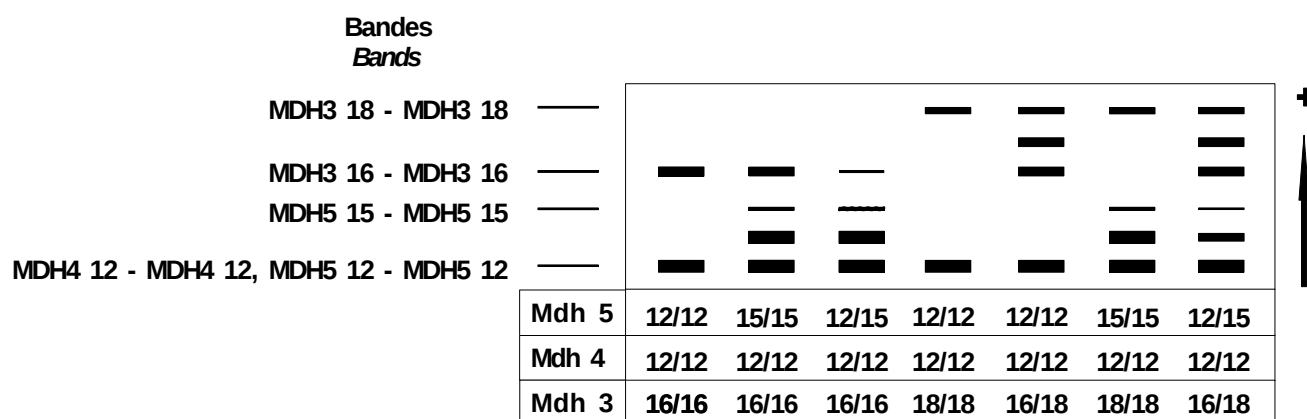
- Alleles 0.5 and 1 from Mdh1 are difficult to discriminate from each other. Therefore, they are scored as identical (note 1). The same is true for alleles 3 and 3.5 from Mdh2 which are scored together (note 1)
- There are interactions between the products of the genes (polypeptide subunits) on the one hand, encoded by Mdh1, Mdh2, Mdh3, and on the other hand, encoded by Mdh4 and Mdh5.

Genotype						Example inbred lines
Mdh1	Mdh2	Mdh3	Mmm	Mdh4	Mdh5	
6/6	6/6	16	M	12	12	A239
6/6	3/3	16	M	12	12	CM7
6/6	6/6	16	M	12	15	F2
6/6	6/6	18	M	12	12	F1444
6/6	3/3	18	M	12	12	CO158
1/1	3/3	16	M	12	12	F252
6/6	4,5/4;5	16	M	12	12	W401

6.1.2 Schematization of the zymogrammes

For the recognition of the alleles at the loci Mdh1, Mdh2 and Mdh4 the SGE at pH 6.5 should be used. For the recognition of the alleles at the loci Mdh3 and Mdh5, a second electrophoresis system should be used : SGE at pH 5.0.

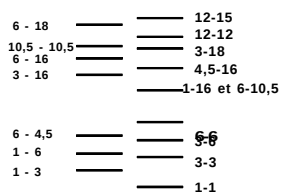
Zymograms of MDH from maize coleoptile in pH 5.0 buffer system:



Some bands which are very faint are drawn in dotted lines. Some bands overlap and cannot be drawn in distinct bands.

Zymograms of MDH from maize coleoptile in pH 6.5 buffer system:

Bandes/Bands



2-15 2-12 2-18 5-16 6 et 6-10,5 6 3 -1																														
Mdh 5	12/12	12/12	15/15	12/12	12/12	12/12	12/12	15/15	12/12	12/12	12/12	12/15	12/12	15/15	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/15				
Mdh 4	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12	12/12				
Mmm	M/M	M/M	M/M	m/m	M/M	M/M	M/M	M/M	m/m	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M	M/M				
Mdh 3	16/16	18/18	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	18/18	18/18	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/18	16/16	16/18				
Mdh 2	6/6	6/6	6/6	6/6	6/6	6/6	6/6	3/3	3/3	3/3	3/4,5	3/4,5	3/3	3/3	3/6	3/6	3/3	3/3	3/3	3/3	4,5/4,5	4,5/6	3/6	3/3	3/6	3/6				
Mdh 1	6/6	6/6	6/6	6/6	10,5/10,5	1/1	1/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	1/6	1/1	6/6	1/6	1/1	6/6	6/6	6/6	6/6	6/6	6/6	6/6				



6.2 Recognition of the alleles encoding IDH

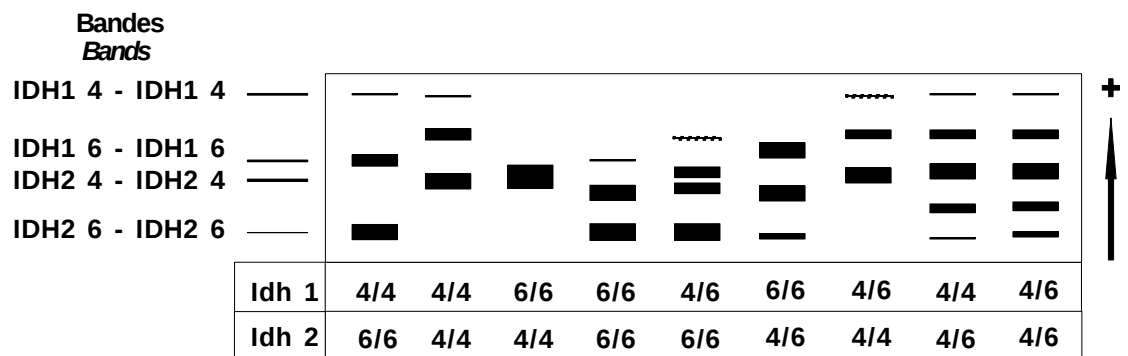
6.2.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal location	Locus	Alleles	
Isocitrate dehydrogenase	Dimeric	8L	Idh1	4, 6	intergenic
(IDH)		6L	Idh2	4, 6	interactions

There are interactions between the products of the genes (polypeptide subunits) encoded by Idh1 and Idh2.

Genotype		Example inbred lines
Idh1	Idh2	
4/4	4/4	F16
4/4	6/6	A632
6/6	4/4	F1110
6/6	6/6	CO158

6.2.2 Schematization of the zymogrammes



Some bands which are very faint are drawn in dotted lines. Some bands overlap and cannot be drawn as distinct bands.

6.3 Recognition of the alleles encoding PGD

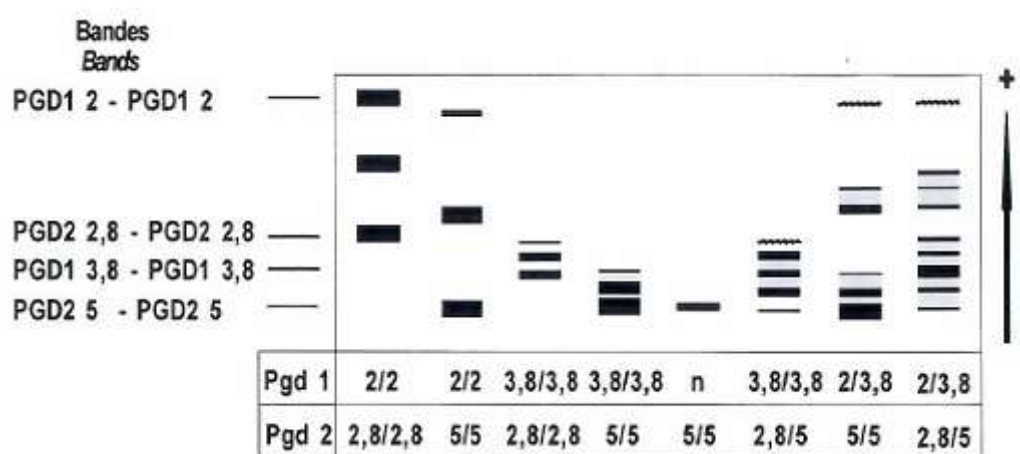
6.3.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal location	Locus	Alleles	
6-phosphogluconate dehydrogenase	Dimeric	6L	Pgd1	2, 3, 8, n	intergenic
(PGD)		3L	Pgd2	2, 8, 5, n	interactions

There are interactions between the products of the genes (polypeptide subunits) encoded by Pgd1 and Pgd2.

Genotype		Example inbred lines
Pgd1	Pgd2	
2/2	5/5	A239
3,8/3,8	2,8/2,8	A632
3,8/3,8	5/5	F2
n/n	5/5	H108

6.3.2 Schematization of the zymogrammes



Some bands which are very faint are drawn in dotted lines. Some bands overlap and cannot be drawn in distinct bands.

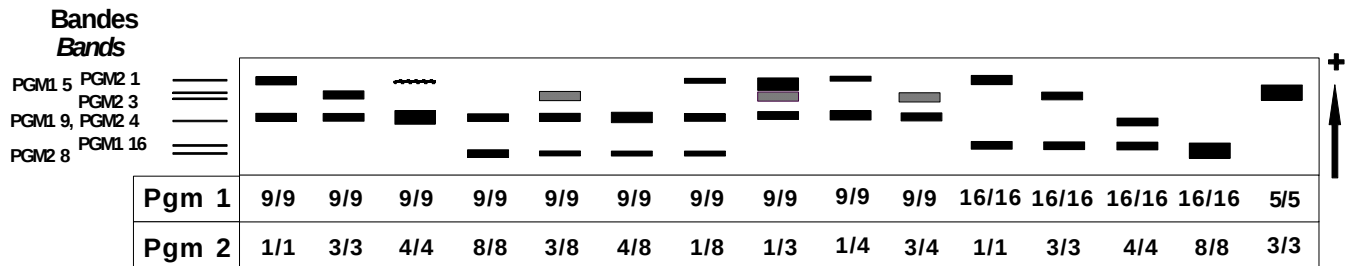
6.4 Recognition of the alleles encoding PGM

6.4.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal location	Locus	Alleles
Phosphoglucomutase	Monomeric	1L	Pgm1	9, 16, 5
				1
(PGM)	Monomeric	5S	Pgm2	3
				4
				8

Genotype		Example inbred lines
Pgm1	Pgm2	
9/9	1/1	F2
9/9	3/3	F16
9/9	4/4	A632
9/9	8/8	MO17

6.4.2 Schematization of the zymogrammes



6.4.3 Distinctness table for the different states of expression at the loci Pgm1 + Pgm2

PGM1			9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	16/16	16/16	16/16	16/16	16/16	16/16	5/5
PGM2			1/1	1/3	3/3	3/4	4/4	1/4	8/8	3/8	4/8	1/8	1/1	1/3	3/3	4/4	8/8	3/3
	Note		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
9/9	1/1	1	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
9/9	1/3	2	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+
9/9	3/3	3	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
9/9	3/4	4	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
9/9	4/4	5	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
9/9	1/4	6	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+
9/9	8/8	7	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
9/9	3/8	8	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
9/9	4/8	9	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
9/9	1/8	10	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
16/16	1/1	11	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
16/16	1/3	12	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
16/16	3/3	13	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
16/16	4/4	14	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
16/16	8/8	15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
5/5	3/3	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

Combinations indicated with “+” can be clearly separated. In general, combinations indicated with “-” cannot be separated.

The notes within grey zones should not be used without knowledge of the parent formula.

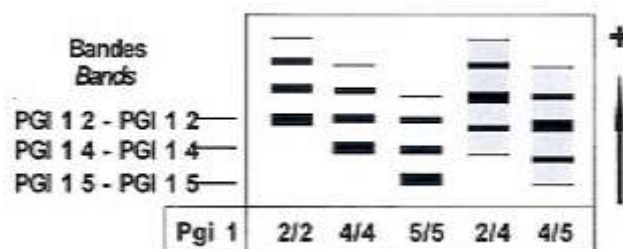
6.5 Recognition of the alleles encoding PGI

6.5.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal location	Locus	Alleles
Phosphoglucosomerase (PGI)	Dimetric	1L	Pgi1	4, 5

Genotype	Example inbred lines
Pgi1	
4/4	A239
5/5	A632

6.5.2 Schematization of the zymogrammes



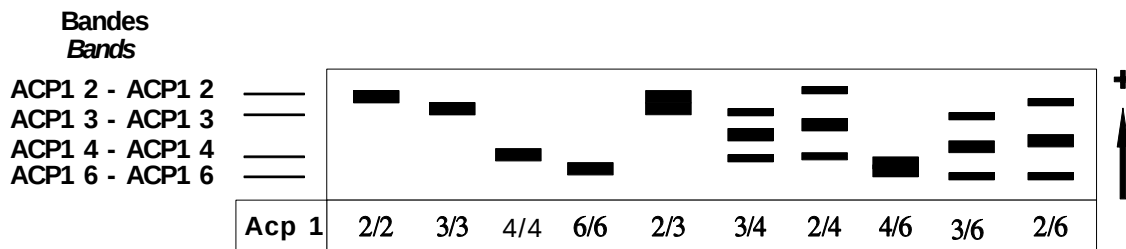
6.6 Recognition of the alleles encoding ACP

6.6.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal location	Locus	Alleles
Acid phosphatase (ACP)	Dimeric	9L	Acp1	2, 3, 4, 6

Genotype	Example inbred lines
Acp1	
2/2	F2
3/3	A239
4/4	A632
6/6	F1444

6.6.2 Schematization of the zymogrammes



Some bands overlap and cannot be drawn as distinct bands.

6.6.3 Distinctness table for the different states of expression at the locus Acp1

ACP1		2/2	2/3	3/3	4/6	4/4	6/6	2/4	2/6	3/4	3/6
	Note	1	2	3	4	5	6	7	8	9	10
2/2	1	-	-	+	+	+	+	+	+	+	+
2/3	2	-	-	-	+	+	+	+	+	+	+
3/3	3	+	-	-	+	+	+	+	+	+	+
4/6	4	+	+	+	-	-	-	+	+	+	+
4/4	5	+	+	+	-	-	+	+	+	+	+
6/6	6	+	+	+	-	+	-	+	+	+	+
2/4	7	+	+	+	+	+	+	-	+	+	+
2/6	8	+	+	+	+	+	+	+	-	+	+
3/4	9	+	+	+	+	+	+	+	+	-	+
3/6	10	+	+	+	+	+	+	+	+	+	-

Combinations indicated with “+” can be clearly separated. In general, combinations indicated with “-” cannot be separated.

The notes within grey zones should not be used without knowledge of the parent formula.

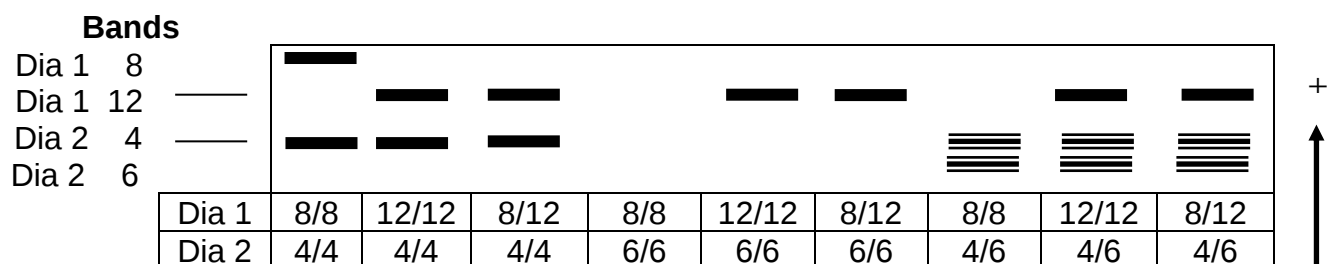
6.7 Recognition of the alleles encoding DIA

6.7.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal location	Locus	Alleles
Diaphorase	Monomeric	2	Dia1	8, 12
(DIA)	Dimetric	1L	Dia2	4, 6

Genotype		Example inbred lines
Dia1	Dia2	
8/8	4/4	F2
12/12	4/4	CO158

6.7.2 Schematization of the zymogrammes



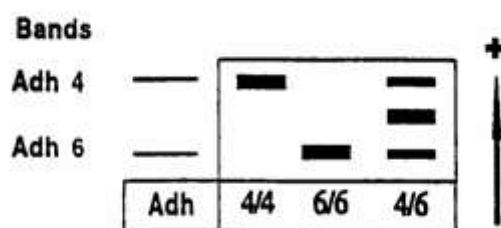
6.8 Recognition of the alleles encoding ADH

6.8.1 Genetic interpretation of the zymogrammes

	Quaternary structure	Chromosomal location	Locus	Alleles
Enzyme				
Alcohol dehydrogenase (ADH)	Dimetric	1L	Adh1	4, 6

Genotype	Example inbred lines
Adh1	
4/4	F1444
6/6	F2

6.8.2 Schematization of the zymogrammes



Description of the example inbred lines

inbred lines	M	M	M	M	M	M	I	I	P	P	P	P	P	A	D	A
lignées endo-	d	d	d	m	d	d	d	d	g	g	g	g	g	c	i	d
games	h	h	h	m	h	h	h	h	d	d	m	m	i	p	a	h
Inzuchtlinien	1	2	3		4	5	1	2	1	2	1	2	1	1	1	1
A239	6/6	6/6	16/16	M/M	12/12	12/12	4/4	4/4	2/2	5/5	9/9	4/4	4/4	3/3	8/8	4/4
A632	6/6	6/6	16/16	M/M	12/12	12/12	4/4	6/6	3,8/3,8	2,8/2,8	9/9	4/4	5/5	4/4	8/8	4/4
CM7	6/6	3/3	16/16	M/M	12/12	12/12	4/4	6/6	3,8/3,8	5/5	9/9	3/3	4/4	4/4	12/12	4/4
CO158	6/6	3/3	18/18	M/M	12/12	12/12	6/6	6/6	3,8/3,8	5/5	9/9	4/4	4/4	4/4	12/12	4/4
F1110	6/6	3/3	16/16	M/M	12/12	12/12	6/6	4/4	3,8/3,8	5/5	9/9	3/3	4/4	3/3	8/8	4/4
F1444	6/6	6/6	18/18	M/M	12/12	12/12	4/4	6/6	3,8/3,8	5/5	9/9	3/3	4/4	6/6	8/8	4/4
F16	1/1	3/3	16/16	M/M	12/12	12/12	4/4	4/4	3,8/3,8	5/5	9/9	3/3	4/4	2/2	8/8	4/4
F2	6/6	6/6	16/16	M/M	12/12	15/15	4/4	4/4	3,8/3,8	5/5	9/9	1/1	4/4	2/2	8/8	6/6
F252	1/1	3/3	16/16	M/M	12/12	12/12	4/4	4/4	3,8/3,8	5/5	9/9	4/4	4/4	3/3	12/12	4/4
H108	6/6	6/6	16/16	M/M	12/12	12/12	4/4	4/4	n/n	5/5	9/9	8/8	4/4	2/2	8/8	4/4
MO17	6/6	6/6	16/16	M/M	12/12	12/12	4/4	4/4	3,8/3,8	5/5	9/9	8/8	4/4	2/2	8/8	4/4
W401	6/6	4,5/4,5	16/16	M/M	12/12	12/12	4/4	6/6	2/2	5/5	9/9	3/3	4/4	2/2	8/8	4/4

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