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**INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA**

DRAFT

MAIZE

UPOV Code: ZEAAA_MAY

Zea mays L.

*

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by experts from France and Hungary

to be considered by the

Technical Working Party for Vegetables

at its forty-first session to be held in Nairobi, Kenya, from June 11 to 15, 2007

Technical Working Party for Agricultural Crops

at its thirty-sixth session to be held in Budapest, Hungary, from May 28 to June 1, 2007

Alternative Names:*

<i>Botanical name</i>	<i>English</i>	<i>French</i>	<i>German</i>	<i>Spanish</i>
<i>Zea mays</i> L.	Maize	Maïs	Maïs	

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

~~VS: visual assessment by observation of individual 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

Unless otherwise indicated, in the case of inbred lines and single hybrids, all observations on single plants (VS, 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. In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 1.

Unless otherwise indicated, in the case of other types of hybrids and open-pollinated varieties, all observations on single plants (VS, 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. In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 1.

[remark DE: 20 plants could be enough for other types of hybrids. 40 plants is ok for open pollinated varieties]

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.

HU supports this proposal, but French don't agree.

"For sweet corn F1 hybrid varieties, the DUS examination should be undertaken on the hybrid and not on the parent lines using the hybrid formula approach"

[remark DE: proposal of HU is already covered by first sentence: "... prescreening system ... may be established ..."]

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

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:

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 electrophoresis of enzymes).

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.

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 and 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:
Tassel: time of anthesis (characteristic 6)
Ear: anthocyanin coloration of silks (characteristic 14)
Plant: length (characteristic 22)
Ear: type of grain (characteristic 34)
Ear: anthocyanin coloration of glumes of cob (characteristic 39)

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): Segregation in the expression of certain characteristics

MG, MS, VG, ~~VS~~: See Chapter 3.3.2

(a) – (d) See Explanations on the Table of Characteristics in Chapter 8.2

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

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

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielsorten/ Variedades ejemplo	Note/ Nota
1. 1.	VG	First leaf: anthocyanin coloration of sheath	Première feuille: pigmentation anthocyanique de la gaine	Primärblatt: Anthocyan-färbung der Blattscheide		
QN	14 (S)	absent or very weak	nulle ou très faible	fehlend oder sehr gering	0674	1
		weak	faible	gering	MO17	3
		medium	moyenne	mittel	F2	5
		strong	forte	stark	F816	7
		very strong	très forte	sehr stark		9
2. 2. (+)	VG	First leaf: shape of tip	Première feuille: forme du sommet	Primärblatt: Form der Spitze		
PQ	14	pointed	pointu	spitz		1
		pointed to round	pointu à arrondi	spitz bis abgerundet	0674	2
		round	arrondi	abgerundet	F816	3
		round to spatulate	arrondi à spatulé	abgerundet bis stumpf	F259	4
		spatulate	spatulé	stumpf	EP1	5
3. 3. (a)	VG	Foliage: intensity of green color	Feuillage: intensité de la couleur verte	Laub: Intensität der Grünfärbung		
QN	51-59	light	claire	hell	W182E	3
		medium	moyenne	mittel	W117, Empire,	5
		dark	foncée	dunkel	W401, Dessert 73,	7

[remark SK: the intensity can be strongly influenced by the external factors – agrotechnics, fertilizers, physiological factors etc. We do not recommend this characteristic. It is difficult to observe]

[remark UA: we would approve char.3]

[remark HU: We would keep it.]

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
4. 3. (+) (a)	VG	Leaf: angle between blade and stem	Feuille: angle entre le limbe et la tige	Blatt: Winkel zwischen Blattspreite und Stengel		
QN	65-69	very small	très petit	sehr klein		1
		small	petit	klein	A188	3
		medium	moyen	mittel	F66	5
		large	grand	gross	F186	7
		very large	très grand	sehr gross		9
5. 4. (+) (a)	VG	Leaf: attitude of blade	Feuille: port du limbe	Blatt: Haltung der Spreite		
QN	65-69	straight	droit	gerade	WD36	1
		slightly recurved	légèrement incurvé	gering gebogen	A654	3
		recurved	incurvé	gebogen	W117	5
		strongly recurved	fortement incurvé	stark gebogen	W79A	7
		very strongly recurved	très fortement incurvé	sehr stark gebogen		9

~~5. Stem: degree of zig-zag-[deletion of this characteristic]~~

[remark SK: We would like to keep this characteristic, we find it as important for distinctness]

[remark UA: may be deleted]

[remark HU: I am not against to keep it. For some sweet corn var. it is useful]

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
6. 7. (*) (+) (b)	MG	Tassel: time of anthesis	Panicule: époque de floraison mâle	Rispe: Zeitpunkt der männlichen Blüte		
QN	61	very early	très précoce	sehr früh		1
	[Fr 65?]	very early to early	très précoce à précoce	sehr früh bis früh	KW1069, Spirit	2
		early	précoce	früh	F257, Champ	3
		early to medium	précoce à moyenne	früh bis mittel	F259, Centurion	4
		medium	moyenne	mittel	F522, Zenith	5
		medium to late	moyenne à tardive	mittel bis spät	A632,	6
		late	tardive	spät	B73,	7
		late to very late	tardive à très tardive	spät bis sehr spät	AM1513	8
		very late	très tardive	sehr spät		9
7. 8. (+) (b)	VG	Tassel: anthocyanin coloration at base of glume	Panicule: bourrelet juste en-dessous de la glume	Rispe: Anthocyanfärbung an der Basis der Hüllspelze		
QN	65 (S)	absent or very weak	nulle ou très faible	fehlend oder sehr gering	W117	1
		weak	faible	gering	F66	3
		medium	moyenne	mittel	F107	5
		strong	forte	stark	EP1	7
		very strong	très forte	sehr stark		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
8. 9. (b)	VG	Tassel: anthocyanin coloration of glumes excluding base	Panicule: pigmentation anthocyanique des glumes à l'exclusion de la base	Rispe: Anthocyanfärbung der Hüllspelze ohne Basis			
QN	65-69 (S)	absent or very weak	nulle ou très faible	fehlend oder sehr gering		F259	1
		weak	faible	gering		F2	3
		medium	moyenne	mittel		WD36	5
		strong	forte	stark		W79A	7
		very strong	très forte	sehr stark			9
9. 10. (+) (b)	VG	Tassel: anthocyanin coloration of anthers	Panicule: pigmentation anthocyanique des anthères	Rispe: Anthocyanfärbung der Antheren			
QN	65 (S)	absent or very weak	nulle ou très faible	fehlend oder sehr gering		A654	1
		weak	faible	gering		F2	3
		medium	moyenne	mittel		W182E	5
		strong	forte	stark			7
		very strong	très forte	sehr stark			9
10. 12. (* (+) (c)	VG	Tassel: angle between main axis and lateral branches	Panicule: angle entre l'axe central et les ramifications latérales	Rispe: Winkel zwischen der Mittelachse und den Seitenästen			
QN	65-69	very small	très petit	sehr klein			1
		small	petit	klein		F492	3
		medium	moyen	mittel		EP1	5
		large	grand	gross		F186	7
		very large	très grand	sehr gross			9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
11. 13. (* (+ (c)	VG	Tassel: attitude of lateral branches	Panicule: port des ramifications	Rispe: Haltung der Seitenäste			
QN	65-69 (S)	straight	droit	gerade		F257	1
		slightly recurved	légèrement incurvé	gering gebogen		F16	3
		recurved	incurvé	gebogen		W182E	5
		strongly recurved	fortement incurvé	stark gebogen		F66	7
		very strongly recurved	très fortement incurvé	sehr stark gebogen			9
12. 14. (*	MG	Tassel: number of primary lateral branches	Panicule: nombre de ramifications primaires	Rispe: Anzahl der Seitenäste erster Ordnung			
QN	65-75	absent or very few	nul ou très petit	fehlend oder sehr gering		F7	1
		few	petit	gering		F252	3
		medium	moyen	mittel		F244	5
		many	grand	gross		A188	7
		very many	très grand	sehr gross			9

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
13. 15.	MG	Ear: time of silk emergence (50% of plants)	Epi: époque d'apparition des soies (50% des plantes)	Kolben: Zeitpunkt des Erscheinens der Narbenfäden (50% der Pflanzen)		
QN	65	very early	très précoce	sehr früh		1
		very early to early	très précoce à précoce	sehr früh bis früh	KW1069	2
		early	précoce	früh	F257	3
		early to medium	précoce à moyenne	früh bis mittel	F259	4
		medium	moyenne	mittel	F522	5
		medium to late	moyenne à tardive	mittel bis spät	A632	6
		late	tardive	spät	B73	7
		late to very late	tardive à très tardive	spät bis sehr spät	AM1513	8
		very late	très tardive	sehr spät		9

~~16.~~ **Ear: anthocyanin coloration of silks** [*deletion of this characteristic*]

14. 17. (*)	VG	Ear: anthocyanin coloration of silks	Epi: pigmentation anthocyanique des soies	Kolben: Anthocyanfärbung der Narbenfäden		
QN	65 (S)	absent or very weak	absente ou très faible	fehlend oder sehr gering	F7, F195	1
		weak	faible	gering	F257	3
		medium	moyenne	mittel	F244	5
		strong	forte	stark	W401	7
		very strong	très forte	sehr stark		9

[*remark SK: We would like to keep this characteristic and rename it as Ear : intensity of the anthocyanin coloration of silks*]

[*remark UA: it is advisable to remain it included*]

[*remark HU: no need for both char., one is enough*]

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
15. 6. (+)	VG	Stem: anthocyanin coloration of brace roots	Tige: pigmentation anthocyanique des racines d'ancrage	Stängel: Anthocyanfärbung der Stelzwurzeln			
QN	65-75 (S)	absent or very weak	nulle ou très faible	fehlend oder sehr gering		F16	1
		weak	faible	gering		W117	3
		medium	moyenne	mittel		WD36	5
		strong	forte	stark		EP1	7
		very strong	très forte	sehr stark			9
16. 11. (b)	VG	Tassel: density of spikelets	Panicule: densité des épillets	Rispe: Dichte der Ährchen			
QN	61-69	lax	lâche	locker		F16	3
		medium	moyenne	mittel		EP1	5
		dense	compacte	dicht		F259	7
17. 18.	VG	Leaf: anthocyanin coloration of sheath (in middle of plant)	Feuille: pigmentation anthocyanique de la gaine (au milieu de la plante)	Blatt: Anthocyanfärbung der Blattscheide (in der Mitte der Pflanze)			
QN	69-73 (S)	absent or very weak	nulle ou très faible	fehlend oder sehr gering		F252	1
		weak	faible	gering		F107	3
		medium	moyenne	mittel		F257	4
		strong	forte	stark		EP1	7
		very strong	très forte	sehr stark			9

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
18. 18 -(a) (+)	VG	Stem: anthocyanin coloration of internodes	Tige: pigmentation anthocyanique des entre-nœuds	Stängel : Anthocyanfärbung der Internodien		
QN	71-75 (S)	absent or very weak	nulle ou très faible	fehlend oder sehr gering	F259	1
		weak	faible	gering	F816	3
		medium	moyenne	mittel	W79A	5
		strong	forte	stark	F257	7
		very strong	très forte	sehr stark		9
19. 19 (+)	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		
QN	71	very short	très court	sehr kurz		1
		short	court	kurz	EP1	3
		medium	moyen	mittel	F244	5
		long	long	lang	F492	7
		very long	très long	sehr lang		9
20. 20- (*) (+)	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		
QN	71	very short	très court	sehr kurz		1
		short	court	kurz	EP1	3
		medium	moyen	mittel	W182E	5
		long	long	lang	F492	7
		very long	très long	sehr lang		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
21. 21. (c)	MS	Tassel: length of lateral branch	Panicule: longueur du rameau	Rispe: Länge der Seitenäste			
QN	71	very short	très court	sehr kurz			1
		short	court	kurz	EP1		3
		medium	moyen	mittel	A632		5
		long	long	lang	F492		7
		very long	très long	sehr lang			9
22. 22.1 (*)	MS	Plant: length (tassel included)	Plante: longueur (panicule comprise)	Pflanze: Länge (einschliesslich Rispe)			
QN	75	very short	très courte	sehr kurz	F7		1
		very short to short	très courte à courte		W117		3
		short	courte	kurz	F244		5
		short to medium	courte à moyenne		WD36		7
		medium	moyenne	mittel	PR39D23		9
		medium to long	moyenne à longue				11
		long	longue	lang	PR39Y10		13
		long to very long	longue à très longue		PAOLIS		15
		very long	très longue	sehr lang			17

[See graphs from France in Endnote]

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
23. 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 zur Pflanzenlänge			
23. [DE:M S]						
QN 75	very small	très petit	sehr klein			1
	small	petit	klein		F816	3
	medium	moyen	mittel		F252	5
	large	grand	gross		F481	7
	very large	très grand	sehr gross			9
24.	Leaf: width of blade (leaf of upper ear on the widest part)	Feuille: largeur du limbe (partie la plus large de la feuille de l'épi le plus haut)	Blatt: Breite der Spreite (Blatt des obersten Kolbens an der breitesten Stelle)			
24.						
QN 75	very narrow	très étroit	sehr schmal			1
	narrow	étroit	schmal		F16	3
	medium	moyen	mittel		F244	5
	wide	large	breit		W182E	7
	very wide	très large	sehr breit			9
24(a). Sweetcorn varieties only: time of picking maturity (medium milk) [deletion of this characteristic]						
24(b). Sweetcorn varieties only: tiller : length [deletion of this characteristic]						
25.	Peduncle: length	Pédoncule: longueur	Kolbenstiel: Länge			
25.						
QN 75-85	very short	très court	sehr kurz			1
	short	court	kurz		F259	3
	medium	moyen	mittel		A654	5
	long	long	lang		F107	7
	very long	très long	sehr lang			9

[Proposal by BR: Ear: covering of ear by straw – low (one can see the ear), medium (partially covered), high (ear completely covered)]
[Remark HU: I could agree only with the comment. "Ear: covering of ear by straw" which is similar to my earlier to the sweet corns suggested char.: Ear: covering with husks. We could mark as VG.]

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
26. 26. (*) (+) QN	MS 92 sweet-corn 75-80	Ear: length very short short medium long very long	Epi: longueur très court court moyen long très long	Kolben: Länge sehr kurz kurz mittel lang sehr lang	F2 A654 MO17	1 3 5 7 9
27. 27. QN	MS 92 sweet-corn 75-80	Ear: diameter (in middle) very small small medium large very large	Epi: diamètre (au milieu) très petit petit moyen grand très grand	Kolben: Dicke (in der Kolbenmitte) sehr dünn dünn mittel dick sehr dick	F7 W117 F481	1 3 5 7 9
28. 28 (+)	VG 92 sweet-corn 75-80	Ear: shape conical conico-cylindrical cylindrical	Epi: forme conique cylindro-conique cylindrique	Kolben: Form konisch konisch-zylindrisch zylindrisch	F16 F7 F66	1 2 3
28(a). Sweetcorn varieties only: Ear: shape of apex [<i>deletion of this characteristic</i>]						
29. 29. QN	MS 92 sweet-corn 75-92	Ear: number of rows of grain very few few medium many very many	Epi: nombre de rangs très petit petit moyen grand très grand	Kolben: Kornreihenzahl sehr gering gering mittel gross sehr gross	F257 F16 B73	1 3 5 7 9

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
30. 29 (a)-	VG Sweetcorn hybrid only: Ear: number of colors of grains	Maïs doux hybrides seulement: Epi: nombre de couleurs du grain	Nur Zuckermais- und Wachsmaissorten: Kolben: Anzahl der Kornfarben			
QL	75-92 one	une	eine		Jubilee	1
	two	deux	zwei		Serendipity	2
<i>[remark DE : Sweetcorn varieties only]</i>						
<i>[remark FR: it is possible only for hybrid because it is not possible to have two colour for inbred lines]</i>						
<i>[See comments in Endnote]</i>						
31. 29 (b)- (*)	Sweetcorn varieties only: Grain: intensity of yellow color	Maïs doux seulement: Grain: intensité de la couleur jaune	Nur Zuckermaissorten: Korn: Intensität der Gelbfärbung			
QN	75-80 light	claire	hell		Gyöngymazola	3
	medium	moyenne	mittel		Royalty	5
	dark	foncée	dunkel		Kokanee	7
32. 29 (c)- (d)	Sweetcorn varieties only: Grain: length	Maïs doux seulement: Grain: longueur	Nur Zuckermaissorten: Korn : Länge			
QN	75-80 short	courte	kurz		Champ	3
	medium	moyen	mittel		Boston	5
	long	longue	lang		Empire	7
33. 29 (d) (+) (d)	Sweetcorn varieties only: Grain: width	Maïs doux seulement: Grain: largeur	Nur Zuckermaissorten: Korn: Breite			
QN	75-80 narrow	étroit	schmal		Bonus	3
	medium	moyen	mittel		Dorado	5
	broad	large	breit		Mv Aranyos	7

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
34. 30. (* (+ (d)	VG	Ear: type of grain	Epi: type de grain	Kolben: Korntyp		
QL	92 (S)	flint	corné	Hartmais	F2	1
		flint-like	corné à corné-denté	hartmais-ähnlich	F252	2
		intermediate	corné-denté	Zwischentyp	F107	3
		dent-like	corné-denté à denté	zahnmais-ähnlich	A654	4
		dent	denté	Zahnmais	W182E	5
		sweet	sucré	Zuckermais	Jubilee	6
		pop	pop	Popcorn	Iowa Pop	7
				<i>[see endnote]</i>		
35. 29(e). (*	VG	<u>Sweetcorn varieties only:</u> Grain: shrinkage	<u>Maïs doux seulement:</u> Grain: contraction	<u>Nur Zuckermaissorten:</u> Korn:		
		very weak				1
QN	92	weak	faible		Zarja	3
		medium	moyenne		Merkur	5
		strong	forte		Dorado	7
		very strong			Dessert 74	9
					<i>[new proposal from Hungary]</i>	
36. 34(a) (+)	VG	<u>Popcorn varieties only:</u> Type of popped grain	<u>Popcorn seulement:</u> Type de grain éclaté	<u>Nur Popcornsorten:</u> Typ des gepoppten Korns		
QL	93	butterfly	à ailettes	Schmetterlingtyp	Robust 97461	1
		intermediate	intermédiaire	Zwischentyp		2
		globular	globuleux	Kugeltyp	Robust 90252	3
					<i>[An explanation is necessary and how to assess this characteristic]</i>	

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
37. 34. (*)	VG	Ear: main color of top of grain	Epi: couleur principale du sommet du grain	Kolben: Hauptfarbe der Kornkrone		
QL	92 (S)	white	blanc	weiss	A188, Snowbelle	1
		yellowish white	blanc jaunâtre	gelblich weiss		2
		yellow	jaune	gelb	F259,	3
		yellow orange	jaune orangé	gelborange	F2	4
		orange	orange	orange	F257	5
		red orange	rouge orangé	rotorange		6
		red	rouge	rot		7
		purple	pourpre	purpur		8
		brownish	brunatre		Zenith	9
		blue black	noir-bleu	blauschwarz		10
38. 32. (*)?	VG	<u>Excluding sweet corn varieties:</u> Ear: color of dorsal side of grain	<u>A l'exclusion des variétés de maïs doux:</u> Epi: couleur de la face dorsale du grain	Kolben: Farbe der Kornrückseite		
QL	92 (S)	white	blanc	weiss	F481	1
		yellowish white	blanc jaunâtre	gelblich weiss	A188	2
		yellow	jaune	gelb		3
		yellow orange	jaune orangé	gelborange	F66	4
		orange	orange	orange	EP1	5
		red orange	rouge orangé	rotorange		6
		red	rouge	rot		7
		purple	pourpre	purpur		8
		bronwish	brunatre		[DE+UA: ?]	9
		blue black	noir-bleu	blauschwarz		10

~~33.~~ Ear: anthocyanin coloration of glumes of cob [*Deletion of this characteristic and combine with the next one*]

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
39. 34.	VG	Ear: anthocyanin coloration of glumes of cob	Epi : pigmentation anthocyanique des glumes de la rafle	Kolben: anthocyanfärbung der Spelzen der Spindel		
QN	93 (S)	absent or very weak	nulle ou très faible	sehr gering	F2,F257	1
		weak	faible	gering	F252	3
		medium	moyenne	mittel	W117	5
		strong	forte	stark	A632	7
		very strong	très forte	sehr stark		9

8. Explanations on the Table of Characteristics

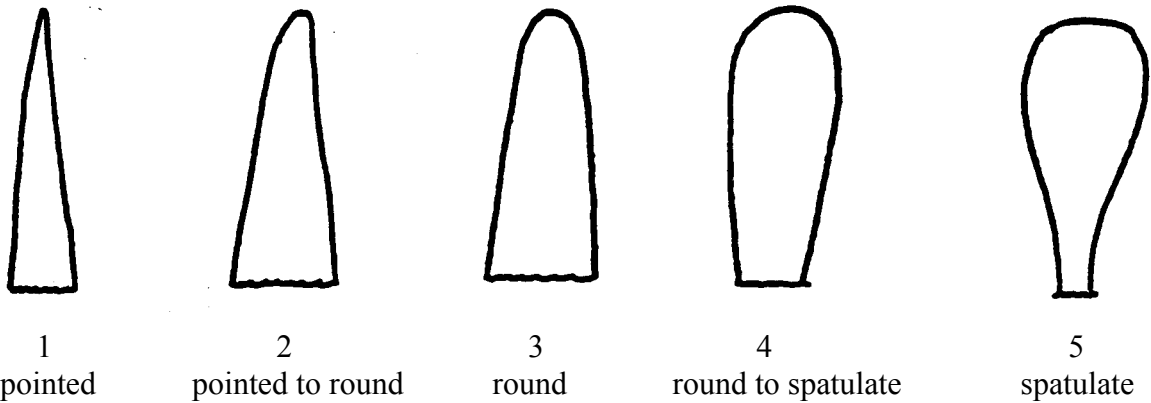
8.1 *Explanations covering several characteristics*

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

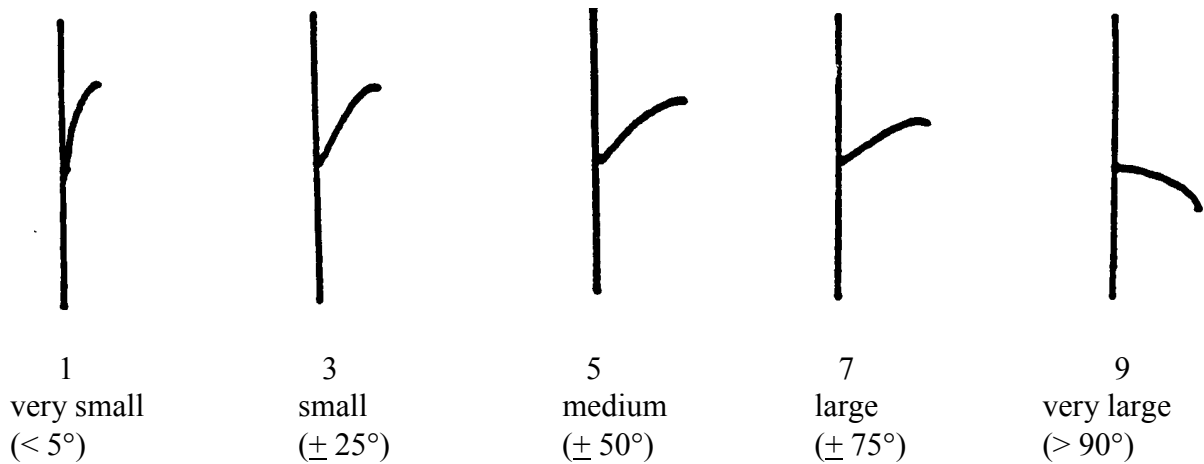
- (a) The observation should be made on the leaf just above upper ear
- (b) The observation should be made in the middle third of main branch.
- (c) The observation should be made on the second branch from the bottom
- (d) The observation should be made in middle third of ear.

8.2 *Explanations for individual characteristics*

Ad. 2: First leaf: shape of tip

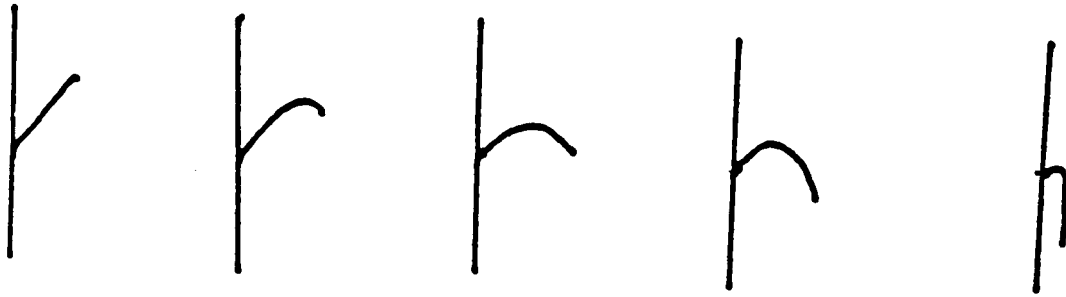


Ad. 4: Leaf: angle between blade and stem



Ad. 5: Leaf: attitude of blade

The observation should be made only on the leaf just above upper ear

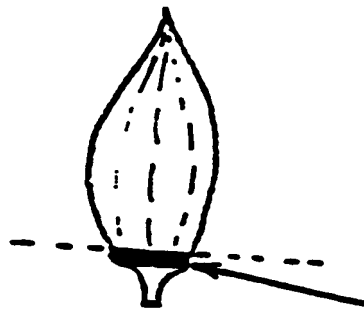


1 straight 3 slightly recurved 5 recurved 7 strongly recurved 9 very strongly

Ad. 7: Tassel: time of anthesis

On main branch with anthers visible on fifty percent of plants

Ad. 7: Tassel: anthocyanin coloration at base of glume

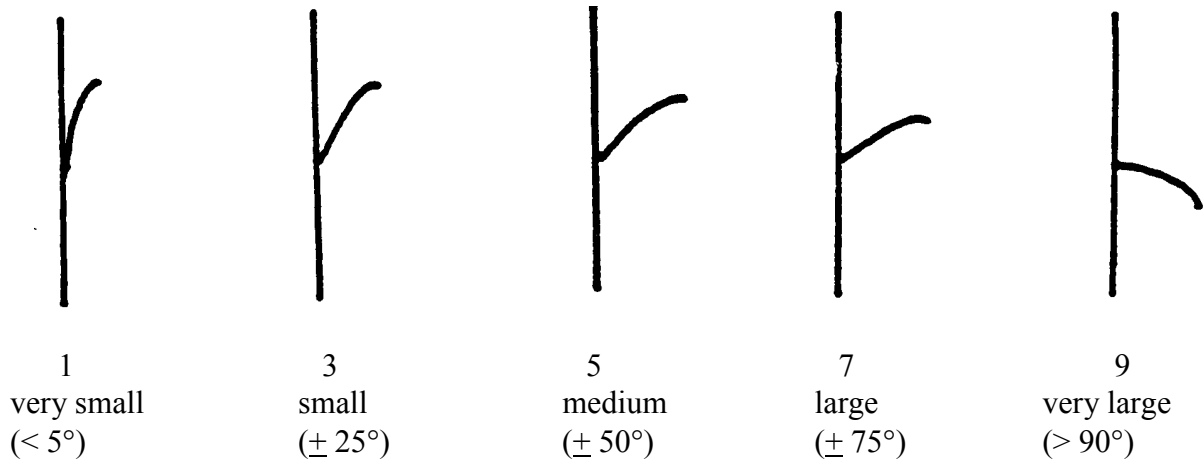


anthocyanin ring

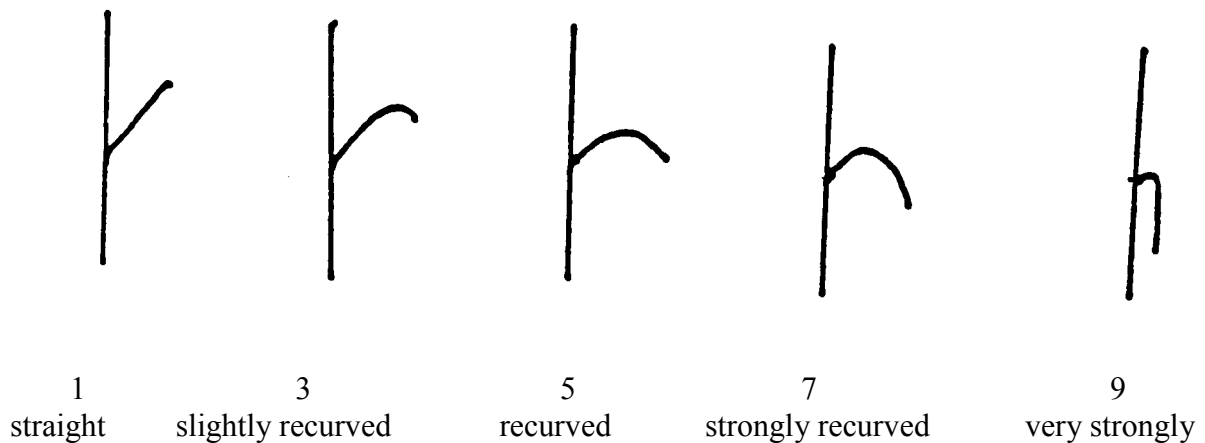
Ad. 9: Tassel: anthocyanin coloration of anthers

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

Ad. 10: Tassel: angle between main branch and lateral branches



Ad. 11: Tassel: attitude of lateral branches



Ad. 15: Stem: anthocyanin coloration of brace roots

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

Ad. 18: Stem: anthocyanin coloration of internode

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

Ad. 19: Tassel: length of main axis above lowest lateral branch



Ad. 20: Tassel: length of main axis above highest lateral branch



Ad. 26: Ear: length (without husks)



Ad. 28: Ear: shape

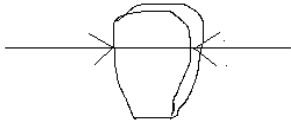


conical

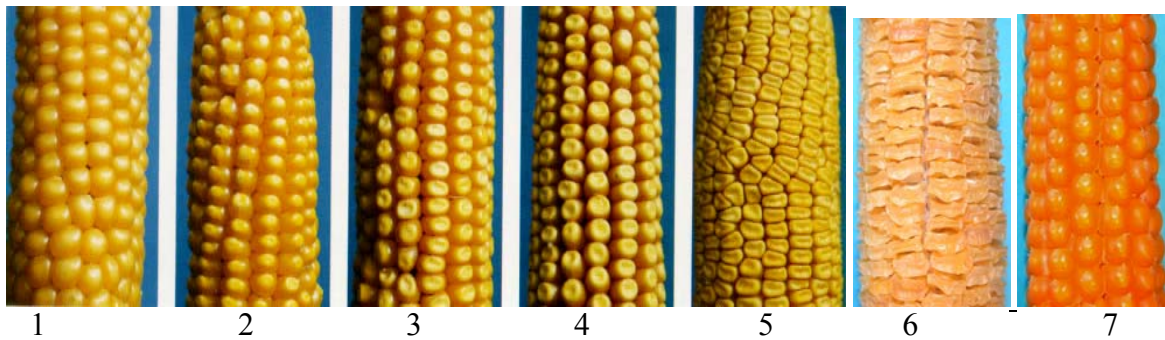
conico-cylindrical

cylindrical

Ad.33: Sweet corn varieties only: grain: width



Ad 34: Ear: type of grain



Ad. 36: Popcorn varieties only: Type of popped grain

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



butterfly



globular

8.3 Decimal Code for the Growth Stages*

The joined document “Phenological growth stages and BBCH-identification keys of Zea mays L” should be considered with regard to a possible inclusion in this TG instead of the table below.

In the new one stage 75 means about 40% dry matter content, in the old one stage 75 was medium milk , which means at sweet corn lower dry matter content (normal sweet about 31% (69% water), super sweet 24% (76% water).

“Kernels in middle of cob yellowish-white” Is this description not confusing for the not yellow colored varieties?

CODE	GENERAL DESCRIPTION		DESCRIPTION
00	<u>Germination</u> Dry seed	<u>Germination</u> Grain sec	<u>Keimung</u> Trockene Saat
12	<u>Seedling growth</u> 2 leaves unfolded	<u>Croissance de la plantule</u> 2 feuilles étalées	<u>Wachstum des Keimlings</u> 2 Blätter entfaltet
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	
(♂,♀)	<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 complète	

	<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
	<u>Dough development</u>	<u>Stade pâteux</u>	<u>Entwicklung der Teigreife</u>
85	Soft dough	Pâteux tendre	weich teigreif
	<u>Ripening</u>	<u>Maturation</u>	<u>Das Reifen</u>
92	Caryopsis hard (can no longer be dented by thumbnail)	Le caryopse est dur (ne peut plus du tout être entamé par l'ongle)	Karyopse hart (nicht mehr mit dem Daumnagel ein- zudellen)
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, 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, 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, Decimal Code for the Growth States of Cereals, EUCARPIA Bulletin No. 7, 1974, pp. 42-52. Die französische Uebersetzung 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.

9. Literature

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Bourgoin-Greneche, M., and Giraud, G., 1994: Technical reference manual for the isoenzymatic analysis of maize. Presentation of the method for scoring the gels and interpretation of the zymogrammes. Ed. GEVES, Guyancourt

Cardy, B.J., and Kanneberg, L.W., 1982: Allozymic variability among maize inbred lines and hybrids: applications for cultivar identification, *Crop Sci.*, 22, 1016-1020

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Newton, K.J., and Schwartz, D., 1980: Genetic basis of the major malate dehydrogenase isozyme in maize. *Genetics*, 95, 425-442

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Smith, J.S.C., and Weissinger, H., 1984: Rapid monitoring of purity in seed lots of hybrid maize: modifications of current technologies. *Maize Genet. Coop. Newsl.*, 58, 103-105

Stuber, C.W., Wendel, J.F., Goodman, M.M., and Smith, J.S.C., 1988: Techniques and scoring procedures for starch gel electrophoresis of enzymes from maize (*Zea mays* L). North Carolina Agricultural Research Service - North Carolina State University, Raleigh

Wendel, J.F., Goodman, M.M., and Stuber, C.W., 1986: Additional mapping of isozyme loci: localization of Acp 4, Dia 2, Adk 1, Tpi 3, and Sad 1. *Maize Gent. Coop. Newsl.* 60, 109-110X.

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	<input type="text" value="Zea mays L."/>	
1.2 Common name	<input type="text" value="Maize"/>	
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"/>	

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
#4. Information on the breeding scheme and propagation of the variety		
4.1 Breeding scheme		
(i)	inbred line/lignée/Inzuchtlinie	[]
(ii)	single-cross hybrid/hybride simple/Einfachkreuzung	
(iii)	three-way cross hybrid/hybride trois voies/Dreiweghybride	
(iv)	double-cross hybrid/hybride double/Doppelhybride	
(v)	open-pollinated variety/variété à fécondation libre/Freiabblühende Sorte	
(vi)	other (indicate formula)/autre (préciser la formule)/Andere (Formel angeben)	[]
<i>(a) Alternative 1</i>		
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)"	[]"
<div style="border: 1px solid black; height: 100px; width: 100%;"></div>		

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

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
<i>(b) Alternative 2</i>		
<p>“Variety resulting from:</p> <p>“4.1.1 Crossing</p> <p>“ (a) controlled cross _____ [] (please state parent varieties)</p> <p>“ (b) partially known cross _____ [] (please state known parent variety(ies))</p> <p>“ (c) unknown cross _____ []</p> <p>“4.1.2 Discovery and development _____ [] (please state where and when discovered and how developed)</p> <p>“4.1.3 Other _____ []” (please provide details)”</p> <div data-bbox="443 1077 1177 1176" style="border: 1px solid black; height: 44px; width: 460px; margin: 10px auto;"></div> <p><i>[DE propose deletion of Alternative2]</i></p>		

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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4.2 Method of propagating the variety

GN 31

The examples below indicate how this section can be formatted and some appropriate terms which can be used:

Example 1

~~“4.2.1 Seed-propagated varieties~~

~~(a) Self-pollination []
(b) Cross-pollination []
(i) population []
(ii) synthetic variety []
(c) Hybrid []
{...see GN 32 for example...}
(d) Other []
(please provide details)~~

~~“4.2.2 Vegetatively propagated varieties~~

~~{...see Example 2...} [.....]~~

~~“4.2.3 Other []”
(please provide details)”~~

[DE propose deletion of chapter 421, 422 and 423]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
<p><i>Example 2</i></p> <p>“4.2.1 Vegetative propagation</p> <p>“(a) cuttings _____ []</p> <p>“(b) <i>in vitro</i> propagation _____ []</p> <p>“(c) other (state method) _____ []</p> <p>“4.2.2 Seed _____ []</p> <p>“4.2.3 Other _____ []” (please provide details)”</p> <div data-bbox="422 887 1177 965" style="border: 1px solid black; height: 35px; width: 473px; margin: 10px auto;"></div> <p><i>[DE propose deletion of this chapter]</i></p>		

TECHNICAL QUESTIONNAIRE

Page {x} of {y}

Reference Number:

GN 32

“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*

“(... female parent line...) x (... male parent line ...)

“*Three-Way Hybrid*

“(... female line ...) x (... male line ...)

“=> single hybrid used as female parent x (... male parent line...)
or (female parent line...) x single hybrid used as male parent

“*Double Hybrid*

“(... female line ...) x (... male line ...)

“=> single hybrid used as female parent “

“(... female line ...) x (... male line ...)”

“=> single hybrid used as male parent”

(single hybrid used as female parent) x (single hybrid used as male parent)

“and should identify in particular:

“(a) any male sterile lines

“(b) maintenance system of male sterile lines.”

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:	
5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).			
Characteristics	Example Varieties	Note	
5.1 Tassel: time of anthesis (on main branch, 50% of plants)			
(6)			
very early		1 []	
very early to early	KW1069, Spirit	2 []	
early	F257, Champ	3 []	
early to medium	F259, Centurion	4 []	
Medium	F522, Zenith	5 []	
medium to late	A632	6 []	
Late	B73	7 []	
late to very late	AM1513	8 []	
very late		9 []	
5.2 Ear: anthocyanin coloration of silks			
(14)			
absent or very weak	F7, F195	1 []	
weak	F257	3 []	
medium	F244	5 []	
strong	W401	7 []	
very strong		9 []	

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
Characteristics	Example Varieties	Note	
5.3 Plant: length (tassel included) (22)			
very short	F7	1 []	
very short to short	W117	3 []	
short	F244	5 []	
short to medium	WD36	7 []	
medium		9 []	
medium to long		11 []	
long		13 []	
long to very long		15 []	
very long		17 []	
5.4 Ear: type of grain (in middle third of ear) (34)			
flint	F2	1 []	
flint-like	F252	2 []	
intermediate	F107	3 []	
dent-like	A654	4 []	
dent	W182E	5 []	
sweet	Jubilee	6 []	
pop	Iowa Pop	7 []	
5.5 Ear: intensity of anthocyanin coloration of glumes of cob (39)			
absent or very weak	F2, F257	1 []	
weak	L274	3 []	
medium	F252	5 []	
strong	W117	7 []	
very strong	A632	9 []	

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
	Characteristics		Example Varieties Note
5.6	<u>Sweetcorn varieties only:</u> Grain: shrinkage		
35	weak		3 []
	medium		5 []
	strong		7 []
5.7	<u>Popeorn varieties only:</u> Type of popped grain		
36	butterfly		1 []
	intermediate		2 []
	globular		3 []
Proposal by HU			
5.8	<u>Sweetcorn varieties only:</u> genetic construction		
	su ₁ (normal sweet varieties)	Jubilee	1 []
	se (sugary enhanced var.)	Gyöngymazsola	2 []
	sh ₂ (super sweet varieties)	Zenith	3 []
	other (give it)		4 []
proposal DE:			
to include the following characteristics:			
Tassel: anthocyanin coloration at base of glume			
Tassel: anthocyanin coloration of glumes excluding base			
Tassel: anthocyanin coloration of anthers			
Ear: color of dorsal side of grain			

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:	
<p>6. Similar varieties and differences from these varieties</p> <p><i>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.</i></p>			
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
GN 33 <i>Example</i>	<i>[e.g. Flower color]</i>	<i>[e.g. orange]</i>	<i>[e.g. orange red]</i>
<p>Comments:</p>			

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
<p>#7. Additional information which may help in the examination of the variety</p> <p>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?</p> <p>Yes [] No []</p> <p>(If yes, please provide details)</p> <p>7.2 Are there any special conditions for growing the variety or conducting the examination?</p> <p>Yes [] No []</p> <p>(If yes, please provide details)</p> <p>7.3 Other information</p>		
<p>8. Authorization for release</p> <p>(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?</p> <p>Yes [] No []</p> <p>(b) Has such authorization been obtained?</p> <p>Yes [] No []</p> <p>If the answer to (b) is yes, please attach a copy of the authorization.</p>		

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

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:												
<p>9. Information on plant material to be examined or submitted for examination.</p> <p>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.</p> <p>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:</p> <table data-bbox="284 819 1407 1079"><tr><td>(a) Microorganisms (e.g. virus, bacteria, phytoplasma)</td><td>Yes []</td><td>No []</td></tr><tr><td>(b) Chemical treatment (e.g. growth retardant, pesticide)</td><td>Yes []</td><td>No []</td></tr><tr><td>(c) Tissue culture</td><td>Yes []</td><td>No []</td></tr><tr><td>(d) Other factors</td><td>Yes []</td><td>No []</td></tr></table> <p>Please provide details for where you have indicated “yes”.</p> <p>.....</p>			(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 []
(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 []												
<p>10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:</p> <p>Applicant's name <input data-bbox="539 1444 1428 1503" type="text"/></p> <p>Signature <input data-bbox="424 1520 984 1579" type="text"/> Date <input data-bbox="1136 1520 1428 1579" type="text"/></p>														

[Annex follows]

ANNEX

11. Additional Useful Explanations

Part I	Introduction	2
Part II	Characteristics Derived by Using Electrophoresis	4
Part III	Description of the SGE Method for the Analysis of Isoenzymes from <i>Zea mays</i> L.	8

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.

For the analysis of enzymes, starch gel electrophoresis is recommended. Polymorphism of enzymes (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).

[remark DE: DE cannot agree to remove heterozygous genotypes and their notes from the table of isoenzyme characteristics. They are needed for the assessment of distinctness of hybrid varieties including variety descriptions. The current guidelines show that in several cases heterozygous and homozygous genotypes have to get the same note as they cannot be distinguished from each other on the zymogrammes. In these cases distinctness of the hybrids cannot be judged from different genotypes of the parental lines.]

[remark HU:]

Hi everybody,

Our time is very short really.

So, I write what I **can** imagine to do and what I **can not** accept at all.

I **can not** imagine at all

- to remove the hybrids from document

- to use only Note or only genotype

Why?

I **can not agree** with Joelle's proposal **to remove the hybrids** (heterozygous genotype) from the document.

I have 3 reasons

- The aim of our work is to check the formula of combination, so we need the description of the hybrids. We started to send a new formula to the breeders that contains both the genotype and the note, as it is in the CPVO document.

We have opportunity to make changes in the document not too frequently, so we have to decide very carefully.

It is better to keep in the document what is not totally good then to lose a lot of information about the hybrids.

Note or genotype?

I have never used the Note, but we put our data into an Excel file that calculate the Note. So, I think until we can not agree upon using or not using the Note, it is the best if both of them remain in the document. We can calculate the Note from the genotype but it causes big problem if there are more genotypes in one Note. Gabriele is in an even worse position because she can not calculate the genotype from the note.

I agree with the following changes

- to use Dia2
- to put the new genotypes into the table

I need Dia2, because we have some varieties containing different Dia2, 4/4 and 6/6 too. It is very easy to distinguish from each other. It makes difficulties to me to describe DIA. I use an arbitrary number to describe them.

I think it is better to use Dia1+Dia2 together, as we do with all enzymes except MDH.

It would be good to put the new genotypes into the table those we could see from the ring test gels using new Note or putting to an old one.

Mdh1: 0.5 and the possible hybrids forms

Mdh2: 3.5 and the possible hybrids forms

Idh1+Idh2: 6/6+4/6 and 4/6+6/6

Pgd1+Pgd2: 2/2+4/4, 3.8/3.8+n/n, n/3.8+5/5 and 2/2+2.8/5

Pgm1+Pgm2: 5/9+3/4

and Dia1+Dia2 as I wrote above.

Proposal for further decisions:

I think we have to check the similar genotypes, having the same Note but are distinguishable, and we have to assign different Note.

I agree with the reunion of ACP and PGM line and hybrid tables. Using the note we can make big mistake to use one table for the lines and another for the hybrids. If we use only the genotypes, we can use the two tables, without risking confusion. However, it is better if we use one table and start the numbering once.

With my best wishes

Agnes

[FR proposal]

As it is difficult (even impossible) to get a complete agreement on a common set of characteristics for inbred lines and hybrids, FR propose to establish two set of characteristics one for inbred lines, and one for hybrids knowing that there is no necessity to compare varieties belonging to these two groups.

In this draft, FR propose the set of characteristics for inbred lines. The set for hybrids must still be elaborated by those applying these characteristics for hybrids. The zymogrames keeps all the cases in order to be able to identify the off types (heterozygous individuals) among inbred lines.

Part II
Characteristics Derived by Using Electrophoresis

English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
40. Allele expression at locus Mdh 1	Expression de l'allèle occupant le locus Mdh 1	Allel-Ausprägung in Locus Mdh 1	Expresión alélica en locus Mdh 1		
Genotype 1/1	Génotype 1/1	Genotyp 1/1	Genotipo 1/1	F252	1
Genotype 6/6	Génotype 6/6	Genotyp 6/6	Genotipo 6/6	A239	2
41. Allele expression at locus Mdh 2	Expression de l'allèle occupant le locus Mdh2	Allel-Ausprägung in Locus Mdh 2	Expresión alélica en locus Mdh 2		
Genotype 3/3	Génotype 3/3	Genotyp 3/3	Genotipo 3/3	F252	1
Genotype 4,5/4,5	Génotype 4,5/4,5	Genotyp 4,5/4,5	Genotipo 4.5/4.5	W401	2
Genotype 6/6	Génotype 6/6	Genotyp 6/6	Genotipo 6/6	A239	3
42. Allele expression at locus Mdh 3	Expression de l'allèle occupant le locus Mdh 3	Allel-Ausprägung in Locus Mdh 3	Expresión alélica en locus Mdh 3		
Genotype 16/16	Génotype 16/16	Genotyp 16/16	Genotipo 16/16	F252	1
Genotype 18/18	Génotype 18/18	Genotyp 18/18	Genotipo 18/18	CO158	2
43. Allele expression at locus Mmm	Expression de l'allèle occupant le locus Mmm	Allel-Ausprägung in Locus Mmm	Expresión alélica en locus Mmm		
Genotype M/M	Génotype M/M	Genotyp M/M	Genotipo M/M	F252	1
Genotype m/m	Génotype m/m	Genotyp m/m	Genotipo m/m	86N42	2
44. Allele expression at locus Mdh 4+ Mdh5	Expression de l'allèle occupant le locus Mdh 4+Mdh5	Allel-Ausprägung in Locus Mdh 4+ Mdh5	Expresión alélica en locus Mdh 4+ Mdh 5		
Genotype 12/12+12/12	Génotype 12/12+12/12	Genotyp 12/12+12/12	Genotipo 12/12+12/12	F252	1
Genotype 12/12+15/15	Génotype 12/12+15/15	Genotyp 12/12+15/15	Genotipo 12/12+15/15	F2	2

English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
45. Allele expression at locus Idh1+Idh2	Expression occupant Idh1+Idh2	de le l'allèle locus	Allel-Ausprägung in Locus Idh1+Idh2	Expresión alélica en locus Idh1+Idh2	
Genotype 4/4+4/4	Génotype 4/4+4/4	Genotyp 4/4+4/4	Genotipo 4/4+4/4	A239	1
Genotype 4/4+6/6	Génotype 4/4+6/6	Genotyp 4/4+6/6	Genotipo 4/4+6/6	CM7	2
Genotype 6/6+4/4	Génotype 6/6+4/4	Genotyp 6/6+4/4	Genotipo 6/6+4/4	F1110	3
Genotype 6/6+6/6	Génotype 6/6+6/6	Genotyp 6/6+6/6	Genotipo 6/6+6/6	CO158	4
46. Allele expression at locus Pgd1+Pgd2	Expression occupant Pgd1+Pgd2	de le l'allèle locus	Allel-Ausprägung in Locus Pgd1+Pgd2	Expresión alélica en locus Pgd1+Pgd2	
Genotype 2/2+5/5	Génotype 2/2+5/5	Genotyp 2/2+5/5	Genotipo 2/2+5/5	W401	1
Genotype 2/2+2.8/2.8	Génotype 2/2+2.8/2.8	Genotyp 2/2+2.8/2.8	Genotipo 2/2+2.8/2.8	SK203	2
Genotype 3.8/3.8+2.8/2.8	Génotype 3.8/3.8+2.8/2.8	Genotyp 3.8/3.8+2.8/2.8	Genotipo 3.8/3.8+2.8/2.8	A632	3
Genotype 3.8/3.8+5/5	Génotype 3.8/3.8+5/5	Genotyp 3.8/3.8+5/5	Genotipo 3.8/3.8+5/5	F252	4
Genotype n/n+5/5	Génotype n/n+5/5	Genotyp n/n+5/5	Genotipo n/n+5/5	H108	5

English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
47. Allele expression at locus Pgm1+Pgm2	Expression de l'allèle occupant le locus Pmd1+Pgm2	Allel-Ausprägung in Locus Pgm1+Pgm2	Expresión alélica en locus Pgm1+Pgm2		
Genotype 9/9+1/1	Génotype 9/9+1/1	Genotyp 9/9+1/1	Genotipo 9/9+1/1	F2	1
Genotype 9/9+3/3	Génotype 9/9+3/3	Genotyp 9/9+3/3	Genotipo 9/9+3/3	F16	2
Genotype 9/9+4/4	génotype 9/9+4/4	Genotyp 9/9+4/4	Genotipo 9/9+4/4	A632	3
Genotype 9/9+8/8	Génotype 9/9+8/8	Genotyp 9/9+8/8	Genotipo 9/9+8/8	MO17	4
Genotype 16/16+1/1	Génotype 16/16+1/1	Genotyp 16/16+1/1	Genotipo 16/16+1/1		5
Genotype 16/16+3/3	Génotype 16/16+3/3	Genotyp 16/16+3/3	Genotipo 16/16+3/3	9034	6
Genotype 16/16+4/4	Génotype 16/16+4/4	Genotyp 16/16+4/4	Genotipo 16/16+4/4		7
Genotype 16/16+8/8	Génotype 16/16+8/8	Genotyp 16/16+8/8	Genotipo 16/16+8/8	F492	8
Genotype 5/5+3/3	Génotype 5/5+3/3	Genotyp 5/5+3/3	Genotipo 5/5+3/3	D06	9
47. Allele expression at locus Pgi1	Expression de l'allèle occupant le locus Pgi1	Allel-Ausprägung in Locus Pgi1	Expresión alélica en locus Pgi1		
Genotype 4/4	Génotype 4/4	Genotyp 4/4	Genotipo 4/4	A239	1
Genotype 5/5	Génotype 5/5	Genotyp 5/5	Genotipo 5/5	A632	2
48. Allele expression at locus Acp1	Expression de l'allèle occupant le locus Acp1	Allel-Ausprägung in Locus Acp1	Expresión alélica en locus Acp1		
Genotype 2/2	Génotype 2/2	Genotyp 2/2	Genotipo 2/2	F2	1
Genotype 3/3	Génotype 3/3	Genotyp 3/3	Genotipo 3/3	A239	2
Genotype 4/4	Génotype 4/4	Genotyp 4/4	Genotipo 4/4	A632	3
Genotype 6/6	Génotype 6/6	Genotyp 6/6	Genotipo 6/6	F1444	4

English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
49. Allele expression at locus Dia1	Expression de l'allèle occupant le locus Dia1	Allel-Ausprägung in Locus Dia1	Expresión alélica en locus Dia1		
Genotype 8/8	Génotype 8/8	Genotyp 8/8	Genotipo 8/8	F2	1
Genotype 12/12	Génotype 12/12	Genotyp 12/12	Genotipo 12/12	CO158	2
50. Allele expression at locus Dia2	Expression de l'allèle occupant le locus Dia2	Allel-Ausprägung in Locus Dia2	Expresión alélica en locus Dia2		
Genotype 4/4	Génotype 4/4	Genotyp 4/4	Genotipo 4/4	F2	1
Genotype 6/6	Génotype 6/6	Genotyp 6/6	Genotipo 6/6	34M838 / FR7905	2
51. Allele expression at locus Adh1	Expression de l'allèle occupant le locus Adh1	Allel-Ausprägung in Locus Adh1	Expresión alélica en locus Adh1		
Genotype 4/4	Génotype 4/4	Genotyp 4/4	Genotipo 4/4	F1444	1
Genotype 6/6	Génotype 6/6	Genotyp 6/6	Genotipo 6/6	F2	2

Part III

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

1. Number of coleoptiles per test

- for checking formula: at least 4 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 4 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 p.H 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 isoenzymes

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

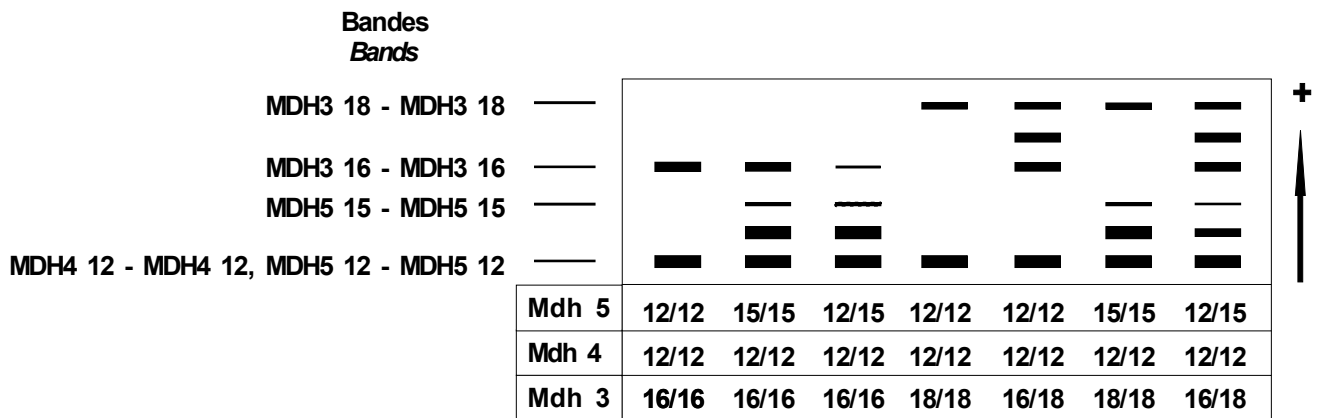
- Alleles 0.5 and 1 from Mdh1 are difficult to discriminate from each other. Therefore, they are scored as identical (note 1). The same is true for alleles 3 and 3.5 from Mdh2 which are scored together (note 1)
- There are interactions between the products of the genes (polypeptide subunits) on the one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

6.2 Recognition of the alleles encoding IDH

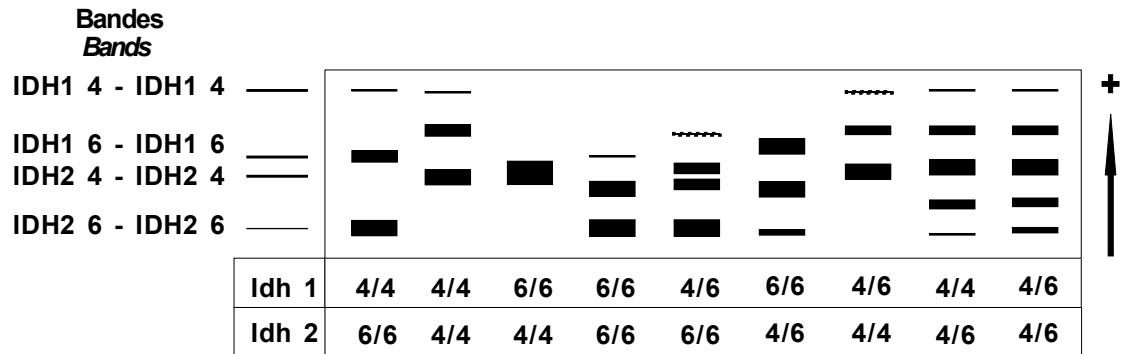
6.2.1 Genetic interpretation of the zymogrammes

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

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

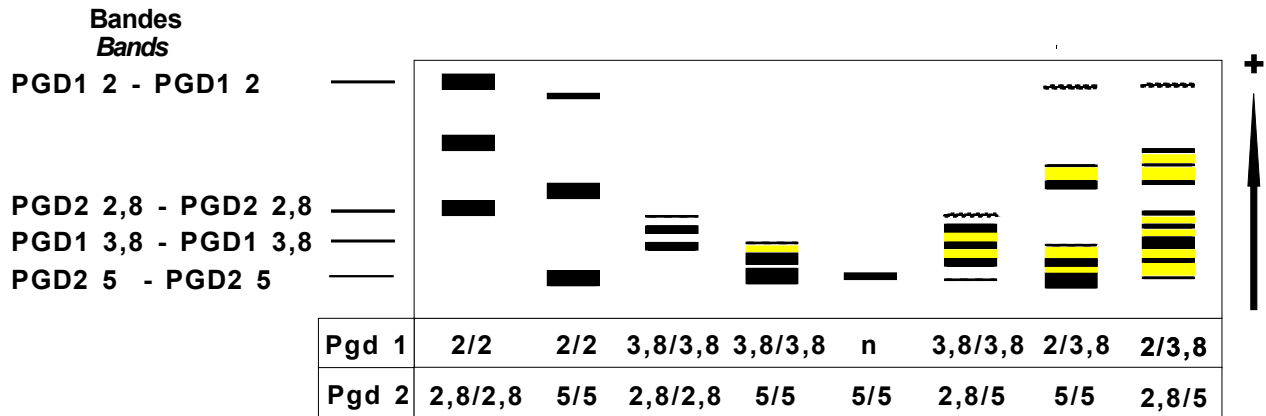
6.3.1 Genetic interpretation of the zymogrammes

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

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.

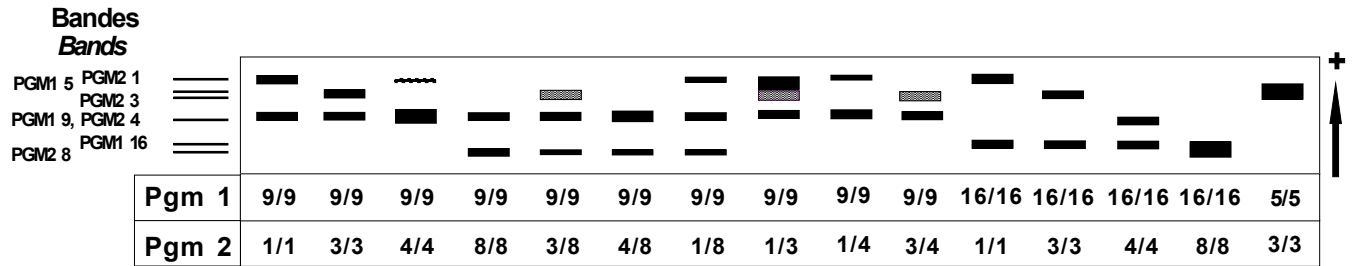
6.4 Recognition of the alleles encoding PGM

6.4.1 Genetic interpretation of the zymogrammes

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



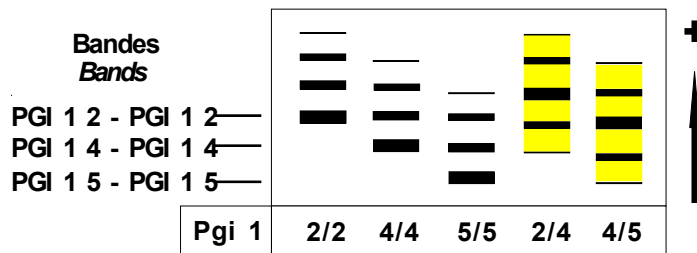
6.5 Recognition of the alleles encoding PGI

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



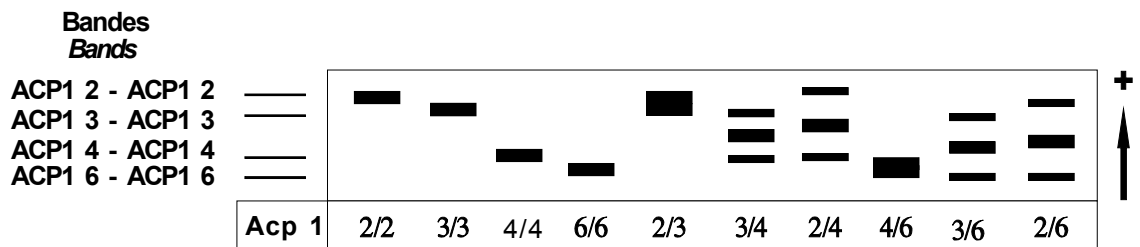
6.6 Recognition of the alleles encoding ACP

6.6.1 Genetic interpretation of the zymogrammes

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

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.

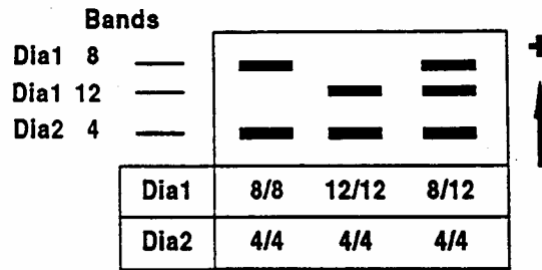
6.7 Recognition of the alleles encoding DIA

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



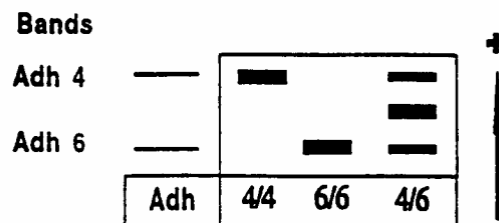
6.8 Recognition of the alleles encoding ADH

6.8.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal location	Locus	Alleles
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

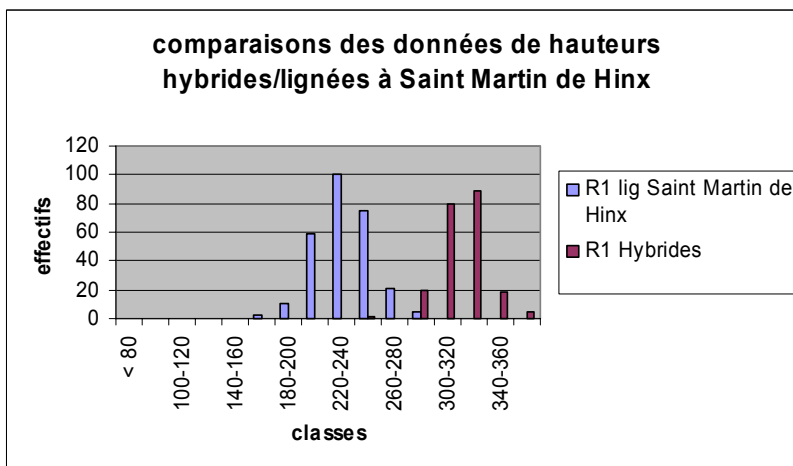
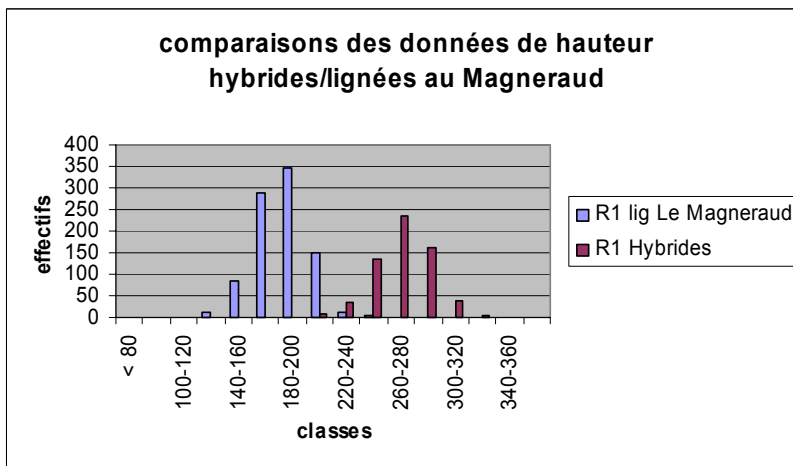
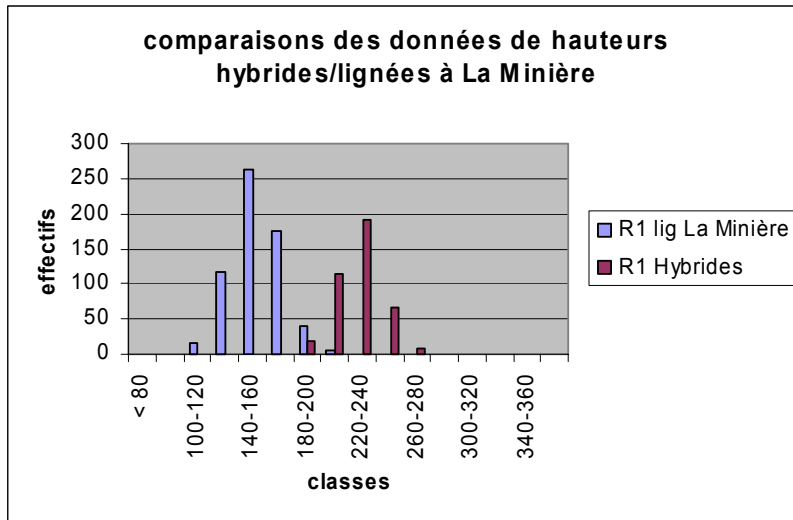


Description of the example inbred lines

inbred lines	M	M	M	M	M	M	I	I	P	P	P	P	P	A	D	A
lignées endo- games	d	d	d	m	d	d	d	d	g	g	g	g	g	c	i	d
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

12. ENDNOTES

Comment on characteristic: 22 Plant: length in France in 2006



In the three locations we have two curves.

Comment on characteristic 30: Sweet corn varieties only: Ear: number of colors of grains

Change of definition in order to be in line with definition of characteristic 31 as one grain (kernel) will have only one color. To explain which combinations of two colors are expected (only combinations with white or also e.g. yellow with yellow orange or orange?). Are there more than two colors possible?

To clarify whether to use 'grain' or 'kernel' (see also for the following characteristics).

Ear: number of kernel colors At sweet corn "kernel" should be the right wording in milky ripened stage. There are varieties with one color (white or yellow) and with two (white and yellow)

Comment on characteristic 34: 'ear: type of grain'

Bundessortenamt

Haßloch,

18.12.2006

309/424

Draft revision UPOV guidelines DUS maize
 characteristic 'ear: type of grain'

current scale of states of expression and notes:

flint	1
flint-like	2
intermediate	3
dent-like	4
dent	5
sweet	6
pop	7

This is a traditional classification with a mixture of descriptions of endosperm type (e.g. flint), crown appearance (e.g. dent) and quality (e.g. sweet and pop). No explanations were given so far. During the last UPOV TWA meeting in Beijing 2006 the inclusion of states 'waxy' and 'flour' was discussed. It became evident that overlapping of aspects like endosperm type and crown appearance in 'waxy' could be a handicap for an understandable scale of states of expression.

It would be highly desirable to have only one scale for all types of grain. From the point of view of Germany all types should be included which have some importance on the market like pop, waxy, flour and sweet. The addition of explanations is necessary to avoid conflicts in case of overlapping aspects. The following proposal can be given:

pop	1	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
flint	2	mostly hard endosperm, round grain, thick layer of hard endosperm

		on crown, larger grains than pop
flint-like	3	mostly hard endosperm, round grain, intermediate layer of hard endosperm on crown
intermediate	4	thin layer of hard endosperm on crown, crown slightly indented
dent-like	5	mostly soft endosperm, crown moderately indented, medium layer of hard endosperm on dorsal side of grain,
dent	6	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
waxy	7	waxy appearance of grain in contrast to all other types of grain, crown appearance can be different
flour	8	completely soft endosperm, grain round or slightly indented on crown
sweet	9	glassy endosperm with very low or no starch content, wrinkled grain

Becher

[FR proposal:]

We prefer to have two characteristics

- one for the morphological aspect "Ear: type of grain"

flint	1	
flint-like	2	
intermediate	3	
dent-like	4	
dent	5	

- the second for nature of the grain "Ear: grain"

normal	1	25% amylose and 75% amylopectine
waxy	2	100% amylopectine
amylose extender	3	75% amylose and 25 % amylopectine
sweet	4	
flour	5	
pop	6	

General remark.

[Remark MX: list of characters that we considered could be to include in the Maiz Guidelines]

	<p>Hoja: Angulo de inserción de las hojas por arriba de la mazorca principal (ángulo formado entre la nervadura central y el eje del tallo).</p> <p>Leaf: angle of insertion of the leaves above upper ear (angle formed between the central vein of the blade and stem)</p>	61	<p>Erecta ≤ 30° <input type="checkbox"/>emirrecta 31-60° Semihorizontal 61-90° Caída > 90°</p>	<p>Erect Semierect Semihorizontal Falling</p>	<p>1 () 2 () 3 () 4 ()</p>	
	<p>Hoja: Angulo de inserción de las hojas debajo de la mazorca principal (igual que en el descriptor número 8)</p> <p>Leaf: angle of insertion of the leaves below upper ear (equal to previous descriptor)</p>	61	<p>Erecta ≤ 30° <input type="checkbox"/>emirrecta 31-60° Semihorizontal 61-90° Caída > 90°</p>	<p>Erect Semierect Semihorizontal Falling</p>	<p>1 () 2 () 3 () 4 ()</p>	
	<p>Hoja: Intensidad de la ondulación del margen laminar (en la hoja de la mazorca principal).</p> <p>Leaf: Intensity of winding of blade margin (in the leaf of principal ear)</p>	61	<p>Ausente o muy débil Débil Media Fuerte Muy fuerte</p>	<p>Absent or very weak Weak Medium Strong Very strong</p>	<p>1 () 3 () 5 () 7 () 9 ()</p>	
	<p>Hoja: Arrugas longitudinales en la hoja de la mazorca principal.</p> <p>Leaf: longitudinal wrinkles in the leaf of principal ear</p>	65	<p>Ausentes Ocasionalmente presentes Siempre presentes</p>	<p>Absents Occasional presents Always presents</p>	<p>1 () 2 () 3 ()</p>	
	<p>Hoja: Coloración de la vaina en la hoja de la mazorca principal.</p> <p>Leaf: coloration of sheath in the leaf of principal ear</p>	65-71	<p>Verde limón Verde (normal) Verde oscuro Verde muy oscuro Morada Roja Café</p>	<p>Pale green Green (normal) Dark green Very dark green Purple Red Brown</p>	<p>1 () 2 () 3 () 4 () 5 () 6 () 7 ()</p>	

	<p>Hoja: Coloración de la aurícula en la hoja de la mazorca principal.</p> <p>Leaf: coloration of the auricle in the leaf of principal ear</p>	65-71	<p>Blanca Verde pálido Roja Café</p>	<p>White Pallid green Red Brown</p>	<p>1 () 2 () 3 () 4 ()</p>	
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	<p>Hoja: Pubescencia sobre el margen de la vaina de la hoja de la mazorca principal.</p> <p>Leaf: pubescence of margin of the sheath in the leaf of principal ear</p>	65-71	<p>Ausente Escasa Intermedia Abundante Muy abundante</p>	<p>Absent Scarce Medium Abundant Very abundant</p>	<p>1 () 3 () 5 () 7 () 9 ()</p>	
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	<p>Tallo: Número de macollos por planta (ahijamiento).</p> <p>Stem: number of secondary stem for plant</p>	65	<p>Ausentes 1 por planta 2-3 por planta 4-5 por planta > 5 por planta</p>	<p>Absent 1 for plant 2-3 for plant 4-5 for plant > 5 for plant</p>	<p>1 () 2 () 3 () 4 () 5 ()</p>	
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	<p>Espiga: Cubrimiento de panoja por hoja bandera, cuando el 50% de plantas se encuentren en floración masculina.</p> <p>Tassel: covering of tassel for flag leaf, when the 50% of plant are in flowering male</p>	65-71	<p>Ausente o muy leve Leve Moderado Casi total</p>	<p>Absent or very light Light Moderate Almost total</p>	<p>1 () 2 () 3 () 4 ()</p>	
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	Espiga: Ramas secundarias. Tassel: secondary branches	65	Ausentes Ocasionalmente presentes Siempre presentes	Absents Occasional presents Always presents	1 () 2 () 3 ()	
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	Jilote: Floración femenina (cuando el 50 % de las plantas muestre estigmas de más de 1 cm de longitud). Indicar la nota correspondiente a los días a floración media en la zona principal de adaptación. T = Trópico B = Bajío VA = Valles Altos VMA = Valles Muy Altos Ear: time of silk emergence (50% of plants)	65	T ()	B ()	VA ()	VMA ()	1 () 2 () 3 () 4 () 5 () 6 () 7 () 8 () 9 ()	- Very early - Very early to early (KW1069) - Early (F257) - Early to medium (F259) - Medium (F522) - Medium to late (A632) - Late (B73) - Late to very late - Very late	
			≤ 45 46-50 51-55 56-60 61-65 66-70 71-75 76-80 > 80		≤ 55 56-61 62-67 68-73 74-79 80-85 86-91 92-97 > 97	≤ 65 66-72 73-79 80-86 87-93 94-100 101-107 108-114 > 114	≤ 75 76-83 84-91 92-99 100-107 108-115 116-123 124-131 > 131		

(*)	Espiga: Floración masculina (cuando el 50% de las plantas se encuentren liberando polen en el tercio medio del eje principal de la espiga). Indicar la nota correspondiente a los días a floración media en la zona principal de adaptación. T = Trópico B = Bajío VA = Valles Altos VMA = Valles Muy Altos. Tassel: time of anthesis (on middle third of main branch, 50% of plants)	65	T ()	B ()	VA ()	VMA ()	1 () 2 () 3 () 4 () 5 () 6 () 7 () 8 () 9 ()	- Very early - Very early to early (KW1069) - Early (F257) - Early to medium (F259) - Medium (F522) - Medium to late (A632) - Late (B73) - Late to very late - Very late	
			≤ 45 46-50 51-55 56-60 61-65 66-70 71-75 76-80 > 80		≤ 55 56-61 62-67 68-73 74-79 80-85 86-91 92-97 > 97	≤ 65 66-72 73-79 80-86 87-93 94-100 101-107 108-114 > 114	≤ 75 76-83 84-91 92-99 100-107 108-115 116-123 124-131 > 131		

	Jilote: Desarrollo de filodios (grado de abundancia y/o desarrollo).	65	Ausente Escaso Moderado Abundante	Absent Scarce Moderate Abundant	1 () 2 () 3 () 4 ()	
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	Ear: unrolling of filodies (degree of abundance and/or unrolling)		Muy abundante	Very abundant	5 ()	
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	Espiga: Longitud del pedúnculo. Es la longitud existente entre el nudo de la hoja bandera y la rama lateral más baja de la espiga (cm). Tassel: length of peduncle. Length between flag leaf nude and lowest side branch (cm)	65	Muy corta ≤ 1.0 Corta 1.1-4.0 4.1-8.0 8.1-12.0 Mediana 12.1-16.0 Larga 16.1-20.0 20.1-24.0 24.1-28.0 Muy larga > 28.0	Very short Short Medium Long Very long	1 () 2 () 3 () 4 () 5 () 6 () 7 () 8 () 9 ()	
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	Planta: Altura de la mazorca desde la superficie del suelo hasta el nudo de inserción de la mazorca principal (cm). Plant: height of ear since surface of soil until the nude of insertion of principal ear (cm)	85	Muy baja ≤ 60 7 80 Baja 81-100 Media 101-120 Alta 121-140 141-160 Muy alta > 160	Very short Short Medium Long Very long	1 () 2 () 3 () 4 () 5 () 6 () 7 ()	
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	Planta: Número de mazorcas por planta, expresado como porcentaje (número de mazorcas entre número de tallos principales por 100). Plant: number of ears for plant; mentioned as percentage (number of ears between number of stems for 100)	92	0- 20 % 21- 40 % 41- 60 % 61- 80 % 81-100 % 101-120 % 121-140 % 141-160 % >160 %		1 () 2 () 3 () 4 () 5 () 6 () 7 () 8 () 9 ()	
--	--	----	--	--	---	--

	Mazorca: Disposición de hileras de granos (en la mazorca principal). Ear: Disposition of rows of grain (in the principal ear)	93	Recta Ligeramente en espiral En espiral Irregular	Straight Slightly in spiral In spiral Irregular	1 () 2 () 3 () 4 ()	
--	--	----	--	--	----------------------------------	--

[Remark HU: "Ear: disposition of rows of grain" is similar to my earlier to the sweet corns suggested char. (Ear: straight of rows) and acceptable with the given state of expressions]

	Mazorca: Forma de la corona (en la mazorca principal). Ear: shape of crown (in the principal ear)	92	Hendida Convexa Puntiaguda	Cracked Convex Pointed	1 () 2 () 3 ()	
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[Remark HU: "Ear: shape of crown" is the same as by me suggested „Ear: shape of apex“ which we deleted because of if the end of the ear is not full with grains than the observation is not possible.]