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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

DRAFT

MAIZE

UPOV Code: ZEAAA_MAY

Zea mays L.

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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-second session, to be held in Cracow, Poland, from June 22 to 27, 2008

and the Technical Working Party for Agricultural Crops (TWA) at its thirty-sixth session to be held in Nelspruit, South Africa, from July 14 to 18, 2008

Botanical name	*English*	*French*	*German*	*Spanish*
Zea mays L.	Maize	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. 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.

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. 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.

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. 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.

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

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 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 (tassel included) (characteristic 24)
(e) Ear: type of grain (characteristic 36)
(f) Excluding sweet corn varieties: Ear: main color of dorsal side of grain (characteristic 40)
(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

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.	**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, Jubilee (SC)	1
		weak	faible	gering		MO17, Puma (SC)	3
		medium	moyenne	mittel		F252, Gyöngymazsola (SC)	5
		strong	forte	stark		F244	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		Empire (SC),F816	
		round to spatulate	arrondi à spatulé	abgerundet bis stumpf		F259, Merkur (SC)	4
		spatulate	spatulé	stumpf		EP1	5
3. ~~**2(a)**~~	**VG**	**Foliage: intensity of green color**	**Feuillage: intensité de la couleur verte**	**Laub: Intensität der Grünfärbung**			
QN	**51-59**	light	claire	hell		W182E	1
		medium	moyenne	mittel		W117, Empire (SC)	2
		dark	foncée	dunkel		GSS 3287 (SC), W401	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
4. **(+)**	**VG**	**Leaf: undulation of margin of blade**	**Feuille: ondulation du bord du limbe**	**Blatt: Wellung des Randes der Spreite**			
QN	**51-59**	absent or very weak	absente ou très faible	fehlend oder sehr gering		F2	1
	(a)	moderate	modérée	mäßig		F252, Puma (SC)	2
		strong	forte	stark		F259, Empire (SC)	3
5. ~~3.~~ **(+)**	**VG**	**Leaf: angle between blade and stem**	**Feuille: angle entre le limbe et la tige**	**Blatt: Winkel zwischen Spreite und Stängel**			
QN	**65-69**	very small	très petit	sehr klein			1
	(a)	small	petit	klein		A188	3
		medium	moyen	mittel		F66, GH 2547 (SC)	5
		large	grand	groß		F186, Spirit (SC)	7
		very large	très grand	sehr groß			9
6. ~~4.~~ **(+)**	**VG**	**Leaf: attitude of blade**	**Feuille: port du limbe**	**Blatt: Haltung der Spreite**			
QN	**65-69**	straight	droit	gerade		WD36	1
	(a)	slightly recurved	légèrement incurvé	gering gebogen		A654, Bonus (SC)	3
		medium recurved	moyennement incurvé	mittel gebogen		W117, Jubilee (SC)	5
		strongly recurved	fortement incurvé	stark gebogen		W79A	7
		very strongly recurved	très fortement incurvé	sehr stark gebogen			9
7.	**VG**	**Stem: degree of zig-zag**	**Tige: degré du zig-zag**	**Stängel: Zickzack-ausprägung**			
QN	**65-69**	absent or very slight	nul ou très faible	fehlend oder sehr gering		F2	1
		slight	faible	mäßig		F186	2
		strong	fort	deutlich		F66	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
8. ~~7.~~ (*) (+)	**MG**	**Tassel: time of anthesis**	**Panicule: époque de floraison mâle**	**Rispe: Zeitpunkt der männlichen Blüte**			
QN	**(b)**	very early	très précoce	sehr früh			1
		very early to early	très précoce à précoce	sehr früh bis früh		KW1069, Spirit (SC)	2
		early	précoce	früh		F257, Champ (SC)	3
		early to medium	précoce à moyenne	früh bis mittel		F259, Centurion (SC)	4
		medium	moyenne	mittel		F522, Zenith (SC)	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
9. ~~8.~~ (*) (+)	**VG**	**Tassel: anthocyanin coloration at base of glume**	**Panicule: bourrelet juste en-dessous de la glume**	**Rispe: Anthocyanfärbung an der Basis der Hüllspelze**			
QN	**65-69 (S)**	absent or very weak	nulle ou très faible	fehlend oder sehr gering		W117, Royalty (SC)	1
	(b)	weak	faible	gering		F66, Boston (SC)	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
10. ~~9.~~	**VG**	**Tassel: anthocyanin coloration of glumes excluding base**	**Panicule: pigmentation anthocyanique des glumes à l'exclusion de la base**	**Rispe: Anthocyan-färbung der Hüllspelze ohne Basis**			
QN	**65-69 (S)**	absent or very weak	nulle ou très faible	fehlend oder sehr gering		F259, Empire (SC)	1
	(b)	weak	faible	gering		F2, Royalty (SC)	3
		medium	moyenne	mittel		WD36, Centurion (SC)	5
		strong	forte	stark		W79A	7
		very strong	très forte	sehr stark			9
11. ~~10.~~ **(+)**	**VG**	**Tassel: anthocyanin coloration of anthers**	**Panicule: pigmentation anthocyanique des anthères**	**Rispe: Anthocyanfärbung der Antheren**			
QN	**(S)**	absent or very weak	nulle ou très faible	fehlend oder sehr gering		A654, Empire (SC)	1
	(b)	weak	faible	gering		F2, Royalty (SC)	3
		medium	moyenne	mittel		W182E, Centurion (SC)	5
		strong	forte	stark			7
		very strong	très forte	sehr stark			9
12. ~~12.~~ **(*) (+)**	**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
	(c)	small	petit	klein		F492	3
		medium	moyen	mittel		EP1, Mv. Aryanos (SC)	5
		large	grand	groß		F186, Bonus (SC)	7
		very large	très grand	sehr groß			9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
13. ~~13.~~ (*) (+)	**VG**	**Tassel: attitude of lateral branches**	**Panicule: port des ramifications**	**Rispe: Haltung der Seitenäste**			
QN	**69 (S)**	straight	droit	gerade		F257, El Toro (SC)	1
	(c)	slightly recurved	légèrement incurvé	gering gebogen		F816 Empire (SC)	3
		medium recurved	moyennement incurvé	mittel gebogen		W182E, Bonus (SC)	5
		strongly recurved	fortement incurvé	stark gebogen		F66	7
		very strongly recurved	très fortement incurvé	sehr stark gebogen			9
14. ~~14.~~ (*)	**MS/VG**	**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, Mv. Aryanos (SC)	3
		medium	moyen	mittel		F244, Kokanee (SC)	5
		many	grand	gross		A188, Zenith (SC)	7
		very many	très grand	sehr groß		Suregold (SC)	9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
15. ~~15.~~ **(+)**	**MG**	**Ear: time of silk emergence**	**Épi: époque d'apparition des soies**	**Kolben: Zeitpunkt des Erscheinens der Narbenfäden**			
QN		very early	très précoce	sehr früh		Mv. Aryanos (SC)	1
		very early to early	très précoce à précoce	sehr früh bis früh		KW1069, Spirit (SC)	2
		early	précoce	früh		F257, Champ (SC)	3
		early to medium	précoce à moyenne	früh bis mittel		F259, Royalty (SC)	4
		medium	moyenne	mittel		F522, Bonus (SC)	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. ~~17.~~ **(*)**	**VG**	**Ear: anthocyanin coloration of silks**	**Épi: pigmentation anthocyanique des soies**	**Kolben: Anthocyanfärbung der Narbenfäden**			
QN	**65 (S)**	absent or very weak	nulle ou très faible	fehlend oder sehr gering		F7, F195, Bonus (SC)	1
		weak	faible	gering		F257, El Toro (SC)	3
		medium	moyenne	mittel		F244, Gyöngymazsola (SC)	5
		strong	forte	stark		W401	7
		very strong	très forte	sehr stark			9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
17. ~~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, Jubilee (SC)	1
		weak	faible	gering		W117, Puma (SC)	3
		medium	moyenne	mittel		WD36, El Toro (SC)	5
		strong	forte	stark		EP1	7
		very strong	très forte	sehr stark			9
18. ~~11.~~ ~~(b)~~	**VG**	**Tassel: density of spikelets**	**Panicule: densité des épillets**	**Rispe: Dichte der Ährchen**			
QN	**61-71**	lax	lâche	locker		F16	3
	(b)	medium	moyenne	mittel		EP1, Royalty (SC)	5
		dense	compacte	dicht		F259, Empire (SC)	7
19. ~~18.~~ (+)	**VG**	**Leaf: anthocyanin coloration of sheath**	**Feuille: pigmentation anthocyanique de la gaine**	**Blatt: Anthocyanfärbung der Blattscheide**			
QN	**71-75 (S)**	absent or very weak	nulle ou très faible	fehlend oder sehr gering		W401, Jubilee (SC)	1
		weak	faible	gering		F107	3
		medium	moyenne	mittel		F257	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
20. ~~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, Jubilee (SC)	1
		weak	faible	gering		F816	3
		medium	moyenne	mittel		W79A	5
		strong	forte	stark		F257	7
		very strong	très forte	sehr stark			9
21. ~~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	**71-75**	very short	très court	sehr kurz			1
		short	court	kurz		EP1	3
		medium	moyen	mittel		F244, Bonus (SC)	5
		long	long	lang		F492, Empire (SC)	7
		very long	très long	sehr lang			9
22. ~~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	**71-75**	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
23. ~~21.~~	**MS**	**Tassel: length of lateral branch**	**Panicule: longueur du rameau**	**Rispe: Länge der Seitenäste**			
QN	**71-75**	very short	très court	sehr kurz			1
	(c)	short	court	kurz		EP1	3
		medium	moyen	mittel		A632	5
		long	long	lang		F492	7
		very long	très long	sehr lang			9
24.1 ~~22.1~~ **(*)**	**MS**	**Inbred lines, sweet and pop only: Plant: length (tassel included)**	**Seulement lignées, sucré et pop: Plante: longueur (panicule comprise)**	**Nur Inzuchtlinien, Zuckermais und Popcorn: Pflanze: Länge (einschliesslich Rispe)**			
QN	**75-85**	very short	très courte	sehr kurz		F7	1
		short	courte	kurz		W117, Spirit (SC)	3
		medium	moyenne	mittel		F244, Puma (SC)	5
		long	longue	lang		WD36, Royalty (SC)	7
		very long	très longue	sehr lang		Enterprise (SC)	9
24.2 (*)	**MS**	**Hybrids and open pollinated varieties only, except sweet and pop: Plant: length: (tassel included)**	**Seulement hybrides et variétés à fécondation libre, excepté sucré et pop: Plante: longueur (panicule comprise)**	**Nur Hybriden und freiabblühende Sorten außer Zuckermais und Popcorn: Pflanze: Länge (einschließlich Rispe)**			
QN	**75-85**	very short	très courte	sehr kurz			1
		short	courte	kurz		PR39D23	3
		medium	moyenne	mittel		PR37Y12	5
		long	longue	lang		DKC5166	7
		very long	très longue	sehr lang			9

[See graphs from France in Endnote]

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
25. ~~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 des obersten Kolbens zur Pflanzenlänge**			
QN	**75-85**	very small	très petit	sehr klein		Gyöngymazsola (SC)	1
		small	petit	klein		F816, Spirit (SC)	3
		medium	moyen	mittel		F252, Royalty (SC)	5
		large	grand	groß		F481	7
		very large	très grand	sehr groß			9
26. ~~24.~~	**MS**	**Leaf: width of blade**	**Feuille: largeur du limbe**	**Blatt: Breite der Spreite**			
QN	**75-85**	very narrow	très étroit	sehr schmal			1
	(a)	narrow	étroit	schmal		F16, Champ (SC)	3
		medium	moyen	mittel		F244, Empire (SC)	5
		wide	large	breit		F481, Centurion (SC)	7
		very wide	très large	sehr breit			9
27. ~~25.~~	**VG**	**Peduncle: length**	**Pédoncule: longueur**	**Kolbenstiel: Länge**			
QN	**75-85**	very short	très court	sehr kurz			1
		short	court	kurz		F259, Centurion (SC)	3
		medium	moyen	mittel		A654, Jubilee (SC)	5
		long	long	lang		F107	7
		very long	très long	sehr lang			9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
28. ~~26.~~ **(*) (+)**	**MS**	**Ear: length**	**Épi: longueur**	**Kolben: Länge**			
QN	**92-93**	very short	très court	sehr kurz			1
	sweet -corn 75-79	short	court	kurz		F2	3
		medium	moyen	mittel		A654, Spirit (SC)	5
		long	long	lang		MO17, Empire (SC)	7
		very long	très long	sehr lang			9
29. ~~27.~~	**MS**	**Ear: diameter (in middle)**	**Épi: diamètre (au milieu)**	**Kolben: Dicke (in der Kolbenmitte)**			
QN	**92-93**	very small	très petit	sehr dünn			1
	sweet -corn 75-79	small	petit	dünn		F7	3
		medium	moyen	mittel		W117	5
		large	grand	dick		F481, Centurion (SC)	7
		very large	très grand	sehr dick		Empire (SC)	9
30. ~~28~~ **(+)**	**VG**	**Ear: shape**	**Epi: forme**	**Kolben: Form**			
QN	**92-93**	conical	conique	konisch		F16, Wombat (SC)	1
	sweet -corn 75-79	conico-cylindrical	cylindro-conique	konisch-zylindrisch		F816, Centurion (SC)	2
		cylindrical	cylindrique	zylindrisch		F66, GH2547 (SC)	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
31. ~~29.~~	**MS**	**Ear: number of rows of grain**	**Épi: nombre de rangs**	**Kolben: Anzahl der Kornreihen**			
QN	**92-93**	very few	très petit	sehr gering			1
	sweet -corn	few	petit	gering		F257	3
	75-93	medium	moyen	mittel		F16, Dessert 73 (SC)	5
		many	grand	groß		B73, Bonus (SC)	7
		very many	très grand	sehr groß			9
32. ~~29 (a).~~	**VG**	**Sweetcorn varieties only: Ear: number of colors of grains**	**Maïs doux variétés seulement: Épi: nombre de couleurs du grain**	**Nur Zuckermais-sorten: Kolben: Anzahl der Kornfarben**			
QL	**75-79**	one	une	eine		Jubilee (SC)	1
	(e)	two	deux	zwei		Serendipity (SC)	2
33. ~~29 (b).~~ **(*)**	**VG**	**Sweetcorn varieties only: Grain: intensity of yellow color**	**Maïs doux variétés seulement: Grain: intensité de la couleur jaune**	**Nur Zuckermais-sorten: Korn: Intensität der Gelbfärbung**			
QN	**75-79**	light	claire	hell		Gyöngymazsola (SC)	3
	(e)	medium	moyenne	mittel		Royalty (SC)	5
		dark	foncée	dunkel		Kokanee (SC)	7
34. ~~29 (c).~~	**VG**	**Sweetcorn varieties only: Grain: length**	**Maïs doux variétés seulement: Grain: longueur**	**Nur Zuckermaissorten: Korn: Länge**			
QN	**75-79**	short	courte	kurz			3
	(d)	medium	moyen	mittel		Boston (SC)	5
		long	longue	lang		GH5704 (SC)	7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
35. ~~29 (d)~~ (+)	**VG**	**Sweetcorn varieties only: Grain: width**	**Maïs doux seulement: Grain: largeur**	**Nur Zuckermais-sorten: Korn: Breite**			
QN	**75-79**	narrow	étroit	schmal		Bonus (SC)	3
	(d)	medium	moyen	mittel		Jubilee (SC)	5
		broad	large	breit		Mv. Aranyos (SC)	7
36. ~~30.~~ (*) (+)	**VG**	**Ear: type of grain**	**Épi: type de grain**	**Kolben: Korntyp**			
QL	**92 (S)**	flint	corné	Hartmais		F2	1
	(d)	flint-like	corné à corné-denté	hartmaisähnlich		F252	2
	(e)	intermediate	corné-denté	Zwischentyp		F107	3
		dent-like	corné-denté à denté	zahnmaisähnlich		A654	4
		dent	denté	Zahnmais		W182E	5
		sweet	sucré	Zuckermais		Jubilee (SC)	6
		pop	pop	Popcorn		Iowa Pop	7
		waxy	waxy	Wachsmais			8
		flour	farineux	Mehlmais			9
				[see endnote]			
37. ~~29 (e).~~ (*) (+)	**VG**	**Sweetcorn varieties only: Ear: shrinkage of top of grain**	**Maïs doux seulement: Épi: contraction du sommet du grain**	**Nur Zuckermais-sorten: Kolben: Schrumpfung der Kornkrone**			
QN	**92**	weak	faible	gering		Zarja (SC)	1
	(d)	medium	moyenne	mittel		Merkur (SC)	3
	(e)	strong	forte	stark		Dessert 73 (SC)	5

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
38. ~~34~~ ~~(a)~~ (+)	**VG**	**Popcorn varieties only: Type of popped grain**	**Popcorn seulement: Type de grain éclaté**	**Nur Popcornsorten: Typ des geplatzten Korns**			
QL	**93**	butterfly	à ailettes	Schmetterlingtyp		Robust 97461	1
		intermediate	intermédiaire	Zwischentyp			2
		globular	globuleux	Kugeltyp		Robust 90252	3
39. ~~31.~~ (*)	**VG**	**Ear: main color of top of grain**	**Épi: couleur principale du sommet du grain**	**Kolben: Hauptfarbe der Kornkrone**			
PQ	**92-93 (S)**	white	blanc	weiß		A188, Snowbelle (SC)	1
	(d)	yellowish white	blanc jaunâtre	gelblich weiß			2
	(e)	yellow	jaune	gelb		F259,	3
		yellow orange	jaune orangé	gelborange		F2, Gyöngymazsola (SC)	4
		orange	orange	orange		F257, GH 2547 (SC)	5
		red orange	rouge orangé	rotorange		Dynasty (SC)	6
		red	rouge	rot			7
		purple	pourpre	purpur			8
		brownish	brunatre	bräunlich		Zenith (SC)	9
		blue black	noir-bleu	blauschwarz			10

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note / Nota
40. ~~32.~~ (*)	**VG**	**Excluding sweet corn varieties: Ear: main color of dorsal side of grain**	**A l'exclusion des variétés de maïs doux: Épi: couleur principale de la face dorsale du grain**	**Außer Zuckermais-sorten: Kolben: Hauptfarbe der Kornrückseite**			
PQ	**92-93 (S)**	white	blanc	weiß		F481	1
	(d)	yellowish white	blanc jaunâtre	gelblich weiß		A188	2
	(e)	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
		brownish	brunatre	bräunlich			9
		blue black	noir-bleu	blauschwarz			10
41. ~~34.~~ (*)	**VG**	**Ear: anthocyanin coloration of glumes of cob**	**Épi: pigmentation anthocyanique des glumes de la rafle**	**Kolben: Anthocyan-färbung der Spelzen der Spindel**			
QN	**93 (S)**	absent or very weak	nulle ou très faible	fehlend oder 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

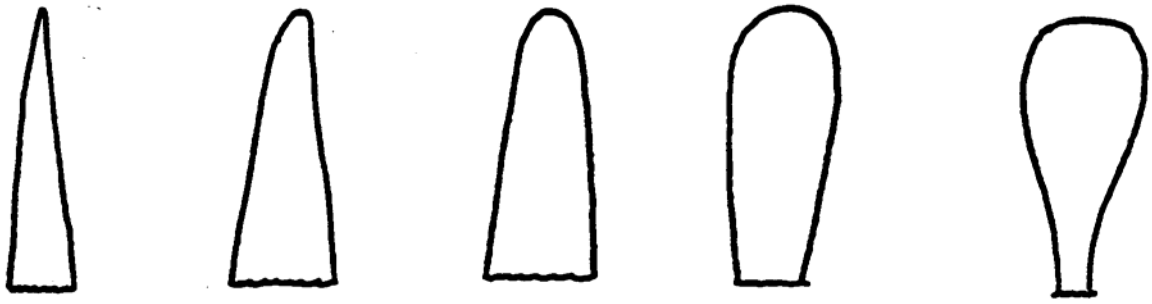
8.1 Explanations covering several characteristics

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 upper well developed ear.
(e) Xenia effect from the neighbor should be avoided.

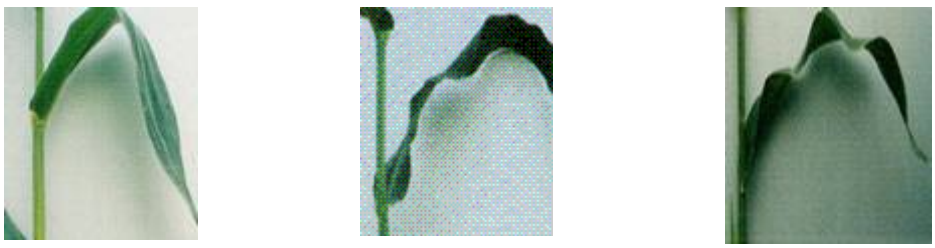
8.2 Explanations for individual characteristics

Ad. 2: First leaf: shape of tip



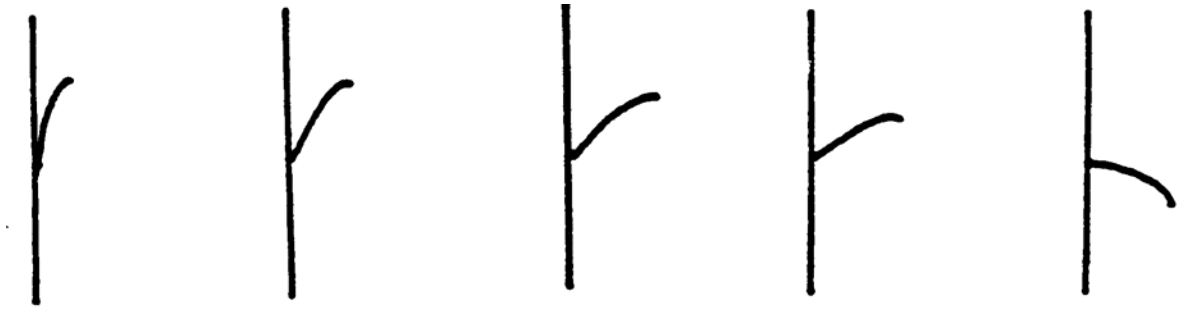
1 pointed | 2 pointed to round | 3 round | 4 round to spatulate | 5 spatulate

Ad. 4: Leaf: undulation of margin of blade



1 absent or very weak | 2 moderate | 3 strong

Ad. 5: Leaf: angle between blade and stem
Ad. 12: Tassel: angle between main axis and lateral branches



1	3	5	7	9
very small (< 5°)	small (± 25°)	medium (± 50°)	large (± 75°)	very large (> 90°)

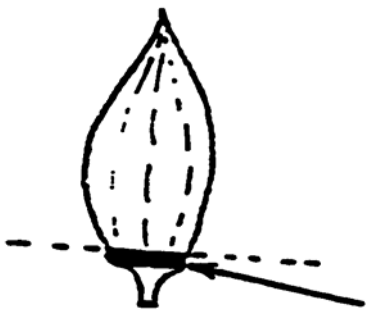
Ad. 6: Leaf: attitude of blade
Ad. 13: Tassel: attitude of lateral branches

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

Ad. 8: Tassel: time of anthesis

On middle third of main branch with anthers visible on 50% of plants

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



base of glume

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

Observation when silk has emerged on 50% of plants.

Ad. 17: Stem: anthocyanin coloration of brace roots

The observation should be made on well developed and fresh roots 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

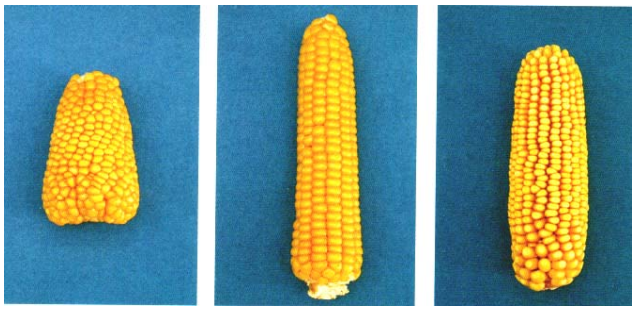
The observation should be made just above insertion point of peduncle of upper ear.

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

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

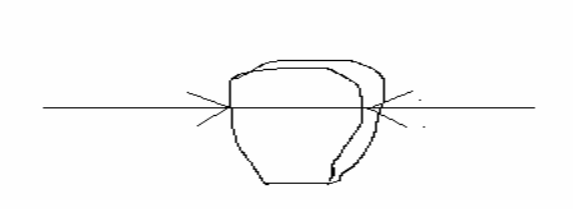
Ad. 28: Ear: length

Ad. 30: Ear: shape

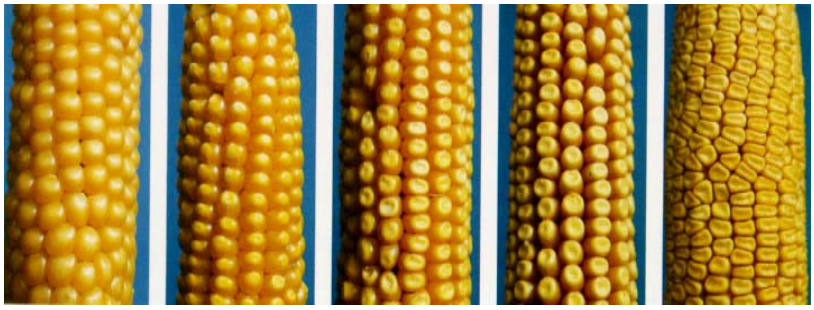


1	2	3
conical	conico-cylindrical	cylindrical

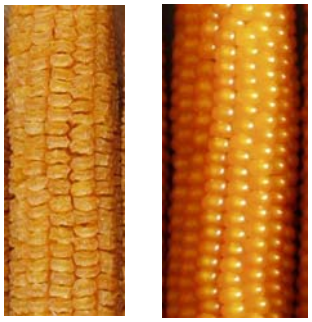
Ad. 35: Sweet corn varieties only: Grain: width



Ad. 36: Ear: type of grain



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



6 sweet | 7 pop | 8 waxy | 9 flour

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	ca. 100 % amylopectine, waxy appearance of grain, pink coloration of endosperm in iodine staining test (blue black coloration of other types of grain).
9	flour	completely soft endosperm, grain round or slightly indented on crown

Iodine staining test

8
waxy

9
other types of grain

Ad. 37: Sweetcorn varieties only: Ear: shrinkage of top of grain

1
weak

3
medium

5
strong

Ad. 38: Popcorn varieties only: Type of popped grain

Ear should be stored 2 or 3 months minimum 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

2
intermediate

3
globular

Decimal Code for the Growth Stages*.

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

CODE	GENERAL DESCRIPTION		DESCRIPTION
	Seedling growth	Croissance de la plantule	Wachstum des Keimlings
14	4 leaves unfolded	4 feuilles étalées	4 Blätter entfaltet
	Tillering	Tallage	Bestockung
	Stem elongation	Elongation de la tige (montaison)	Schossen
	Booting	Gonflement	Schwellstadium
	Inflorescence emergence	Epiaison	Erscheinen des Blütenstands
51 (♂,♀)	Inflorescence just visible	Inflorescence à peine visible	Blütenstand gerade sichtbar
59	Emergence of inflorescence completed	Inflorescence complètement dégagée	Blütenstand vollständig geschoben
(♂,♀)			
	Anthesis	Anthèse	Blüte
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
	Milk development	Stade laiteux	Entwicklung der Milchreife
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
	<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 Daumennagel einzudellen)
93	Caryopsis loosening in daytime	Caryopse se détachant dans la journée	Karyopse tagsüber lockernd

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* 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.

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* 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.

9. Literature

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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:

#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 (indicate formula))	[ ]

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.

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

4.2 Method of propagating the variety

(a)	Self-pollination	[ ]
(b)	Cross-pollination	
	(i) population	[ ]
	(ii) synthetic variety	[ ]
(c)	Hybrid	[ ]
(d)	Other (please provide details)	[ ]

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

Subject to the decision of the competent authority

In the case of hybrid varieties the production scheme should be provided. 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 parent line …) x (… male parent 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 parent line …) x (… male parent line …)

"=> single hybrid used as female parent "

(… female parent line …) x (… male parent 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 female parent lines
(b) maintenance system of male sterile female parent 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 (8)	**Tassel: time of anthesis**		
	very early		1 [ ]
	very early to early	KW1069, Spirit (SC)	2 [ ]
	early	F257, Champ (SC)	3 [ ]
	early to medium	F259, Centurion (SC)	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 (9)	**Tassel: anthocyanin coloration at base of glume**		
	absent or very weak	W117, Royalty (SC)	1 [ ]
	weak	F66, Boston (SC)	3 [ ]
	medium	F107	5 [ ]
	strong	EP1	7 [ ]
	very strong		9[ ]
5.3 (16)	**Ear: anthocyanin coloration of silks**		
	absent or very weak	F7, F195, Bonus (SC)	1 [ ]
	weak	F257, El Toro (SC)	3 [ ]
	medium	F244, Gyöngymazsola (SC)	5 [ ]
	strong	W401	7 [ ]
	very strong		9 [ ]

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

	Characteristics	Example Varieties	Note
5.4I (24.1)	**<u>Inbred lines, sweet and pop only</u>: Plant: length (tassel included)**		
	very short	F7	1 [ ]
	short	W117, Spirit (SC)	3 [ ]
	medium	F244, Puma (SC)	5 [ ]
	long	WD36, Royalty (SC)	7 [ ]
	very long	Enterprise (SC)	9 [ ]
5.4II (24.2)	**<u>Hybrids and open pollinated varieties only, except sweet and pop:</u> Plant: length (tassel included)**		
	very short		1 [ ]
	short	PR39D23	3 [ ]
	medium	PR37Y12	5 [ ]
	long	DKC5166	7 [ ]
	very long		9 [ ]
5.5 (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	7 [ ]
	waxy		8 [ ]
	flour		9 [ ]

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

	Characteristics	Example Varieties	Note
5.6 (40)	**<u>Excluding sweet corn varieties</u>: Ear: main 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[ ]
5.7 (41)	**Ear: anthocyanin coloration of glumes of cob**		
	absent or very weak	F2, F257	1 [ ]
	weak	F252	3 [ ]
	medium	W117	5 [ ]
	strong	A632	7 [ ]
	very strong		9 [ ]

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

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the **similar** variety(ies)	Describe the expression of the characteristic(s) for **your** candidate variety
Example	*[e.g. Flower color]*	*[e.g. orange]*	*[e.g. orange red]*

Comments:

#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 varieties (su1)	Jubilee (SC)	1 [ ]
sugary enhanced varieties (se)	Gyöngymazsola (SC)	2 [ ]
super sweet varieties (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.

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

8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [ ] No [ ]

(b) Has such authorization been obtained?

Yes [ ] No [ ]

If the answer to (b) is yes, please attach a copy of the authorization.

9. Information on plant material to be examined or submitted for examination.

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

(a) Microorganisms (e.g. virus, bacteria, phytoplasma) Yes [ ] No [ ]

(b) Chemical treatment (e.g. growth retardant, pesticide) Yes [ ] No [ ]

(c) Tissue culture Yes [ ] No [ ]

(d) Other factors Yes [ ] No [ ]

Please provide details for where you have indicated “yes”.

……

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

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant's name

Signature | Date

ANNEX

Additional Useful Explanations

TABLE OF CONTENTS PAGE

Part I. Introduction
Part II. Characteristics based on isozyme markers revealed by electrophoresis
Part III. Description of the SGE Method for the Analysis of Isozymes from *Zea mays* L.

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 USING ELECTROPHORESIS

Characteristics			Examples	Note
42	***Allele expression at locus Mdh 1***	Genotype 1/1 Genotype 0.5/0.5 Genotype 0.5/1	F252 R3126 KW 5361 xKW 5454	1
		Genotype 1/6 in interaction with allele 6 of Mdh 2	Tau	
		Genotype 0.5/1 in interaction with allele 6of Mdh 2	Clarica	2
		Genotype 6/6	A239	
		Genotype 1/6 but not in interaction with allele 6 of Mdh 2	Marshall	3
		Genotype 0.5/6 but not in interaction with allele 6 of Mdh 2	DK231	

43	***Allele expression at locus Mdh 2***	Genotype 3/3 Genotype 3.5/3.5 Genotype 3/3.5 Genotype 3/4.5	F252 R3126 Limit, DK 231 Robin	1
		Genotype 4.5/4.5	W401	2
		Genotype 6/6	A239	3
		Genotype 3/6 Genotype 3.5/6	Azur Clarica	4
		Genotype 4.5/6		5
44	***Allele expression at locus Mdh 3***	Genotype 16/16	F252	1
		Genotype 18/18	Co 158	2
		Genotype 16/18	Figaro	3
45	***Allele expression at locus Mmm***	Genotype M/M Genotype M/m	F252	1
		Genotype m/m	86 N 42	2
46	***Allele expression at loci Mdh 4 + Mdh 5***	Genotype 12/12 +12/12	F252	1
		Genotype 12/12 + 15/15 Genotype 12/12 + 12/15	F2 Robin	2

47	***Allele expresion at loci Idh1 + Idh 2***	Genotype 4/4 + 4/4 Genotype 4/6 + 4/4	A239	1
		Genotype 4/4 + 6/6	CM7	2
		Genotype 6/6 + 4/4	F1110	3
		Genotype 6/6 +6/6	Co158	4
		Genotype 4/4 + 4/6 Genotype 4/6 + 4/6	Axon Loft	5
		Genotype 6/6 + 4/6		6
		Genotype 4/6 + 6/6	Bonny	7
48	***Allele expression at loci Pgd 1 + Pgd2***	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 Génotype 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
49.1	***Inbred lines only:*** ***allele expression at loci*** ***Pgm 1 + Pgm2***	Genotype 9/9 + 1/1	F2	1
		Genotype 9/9 + 3/3	F16	2
		Genotype 9/9 + 4/4	A632	3
		Genotype 9/9 + 8/8	Mo17	4
		Genotype 16/16 + 1/1		5
		Genotype 16/16 + 3/3	9034	6
		Genotype 16/16 +4/4		7
		Genotype 16/16 + 8/8	F 492	8
		Genotype 5/5+3/3	D 06	9

49.2	***Hybrids and open-pollinated varieties only:***	Genotype 9/9 + 1/1 Genotype 9/9 + 1/3	Robin	
	allele expression at loci	Genotype 9/9 + 3/3		
	Pgm 1 + Pgm2	Genotype 9/9 + 3/4	Figaro	
		Genotype 9/9 + 4/4		1
		Genotype 9/9 + 1/4		
		Genotype 16/16 + 4/4		
		Genotype 9/9 + 8/8	Axon	
		Genotype 9/9 + 3/8		
		Genotype 9/9 + 4/8		2
		Genotype 9/9 + 1/8	Occitan	3
		Genotype 16/16 + 1/1		
		Genotype 16/16 + 1/3		4
		Genotype 16/16 + 3/3		
		Genotype 16/16 + 8/8		5
50	***Allele expression at locus***	Genotype 4/4	A239	1
	Pgi 1	Genotype 5/5	A632	2
		Genotype 4/5	Artist	3
51.1	***Inbred lines only:***	Genotype 2/2		
	allele expression at locus		F2	1
	Acp1	Genotype 3/3	A239	2
		Genotype 4/4	A632	3
		Genotype 6/6	F1444	4

51.2	***For hybrids and open-***	Genotype 2/3	Azur	1
	pollinated varieties only:	Genotype 2/2		
	Allele expression at locus Acp1	Genotype 3/3		
		Genotype 4/6	Contessa	2
		Genotype 4/4		
		Genotype 6/6		
		Genotype 2/4	Occitan	3
		Genotype 2/6		4
		Genotype 3/4	Marshall	5
		Genotype 3/6		6
52	***Allele expression at locus Dia 1***	Genotype 8/8	F2	1
		Genotype 12/12	Co158	2
		Genotype 8/12	Bastion	3
53	***Allele expression at locus***	Genotype 4/4	F2	1
	Dia2	Genotype 6/6	34 M838	2
		Genotype 4/6	31 N 6	3
54	***Allele expression at locus Adh 1***	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 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 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 1 with de-ionised water

4.2.1.2 Running buffer: 0. 072 ML-histidine-citrate pH 6.5
(Stock solution diluted 1 in 5)
400 ml stock solution (4.2.1.1) made up to 2 1 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-HC1 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-HC1 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-HC1 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 adge 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	Dimeric	3L	Mdh3	16; 18	interactions
(MDH)		1L	Mmm	M; m	
		1L	Mdh4	12	intergenic
		5S	Mdh5	12; 15	interactions

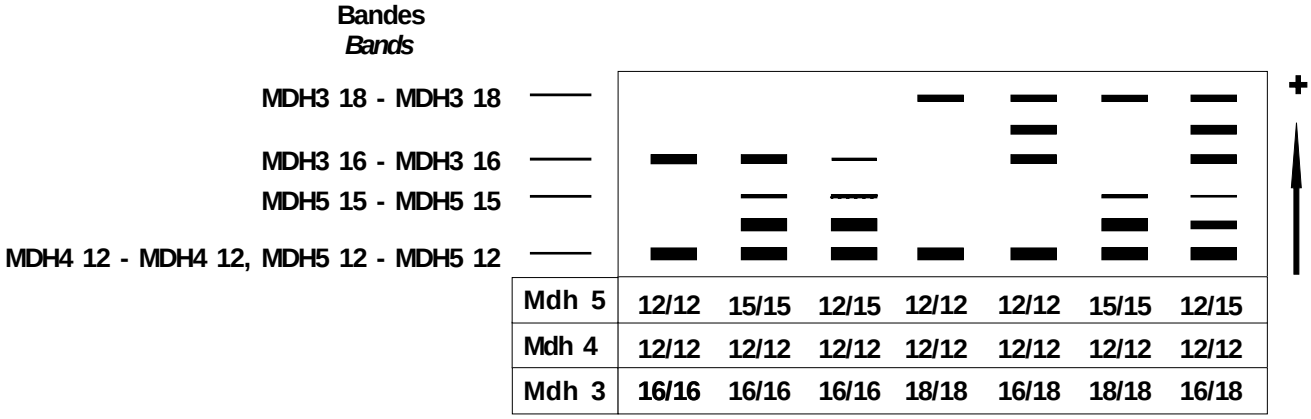
- Alleles 0.5 and 1 from Mdh1 are difficult to discriminate from each other. Therefore, they are scored as identical (note 1). The same is true for alleles 3 and 3.5 from Mdh2 which are scored together (note 1)
- There are interactions between the products of the genes (polypeptide subunits) on the one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

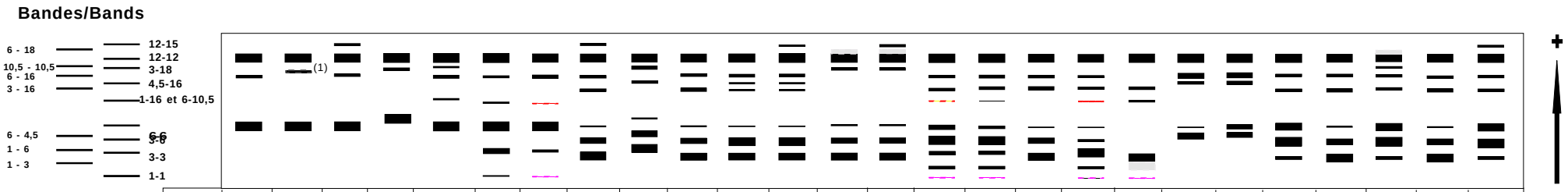
Zymograms of MDH from maize coleoptile in pH 5.0 buffer system:



Mdh 5	**12/12**	**15/15**	**12/15**	**12/12**	**12/12**	**15/15**	**12/15**
Mdh 4	**12/12**	**12/12**	**12/12**	**12/12**	**12/12**	**12/12**	**12/12**
Mdh 3	**16/16**	**16/16**	**16/16**	**18/18**	**16/18**	**18/18**	**16/18**

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:



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	12/12	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	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	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	16/16	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	4,5/4,5	4,5/6	3/6	3/3	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	6/6

6.2 Recognition of the alleles encoding IDH

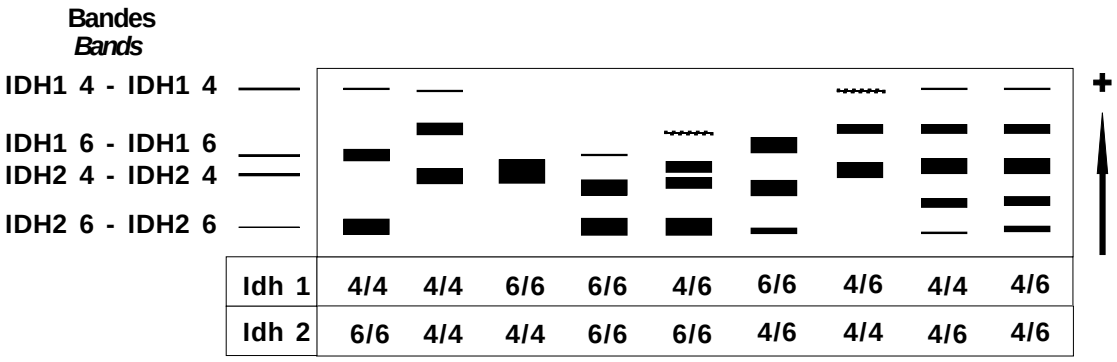
6.2.1 Genetic interpretation of the zymogrammes

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

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

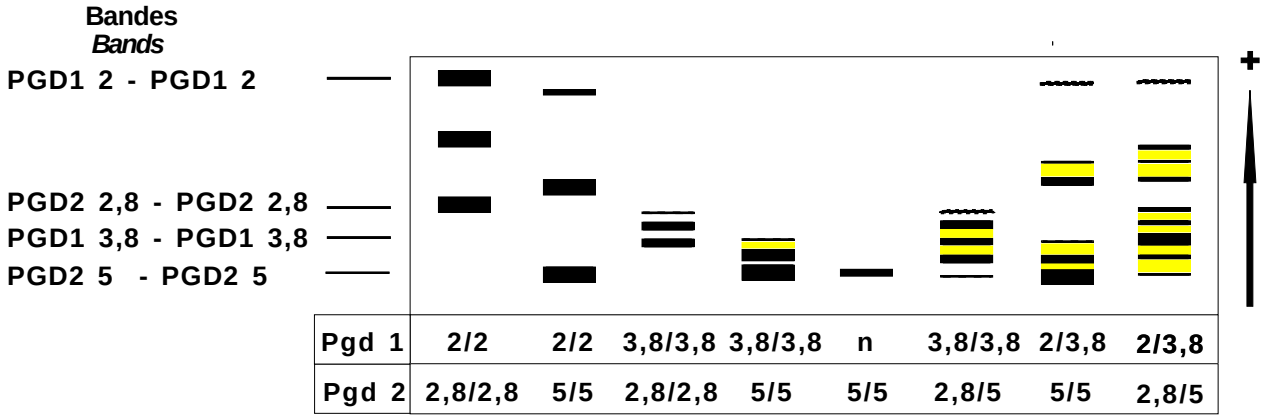
6.3.1 Genetic interpretation of the zymogrammes

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

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.

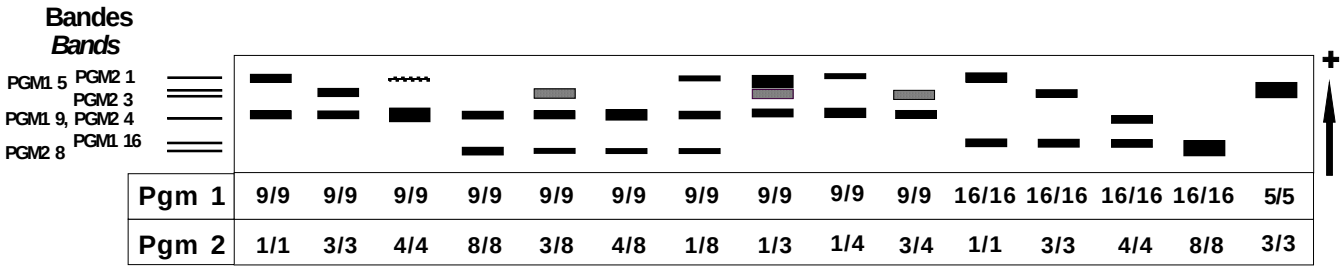
6.4 Recognition of the alleles encoding PGM

6.4.1 Genetic interpretation of the zymogrammes

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



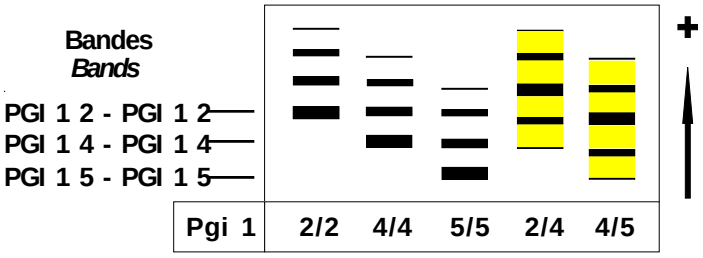
6.5 Recognition of the alleles encoding PGI

6.5.1 Genetic interpretation of the zymogrammes

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

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

6.5.2 Schematization of the zymogrammes



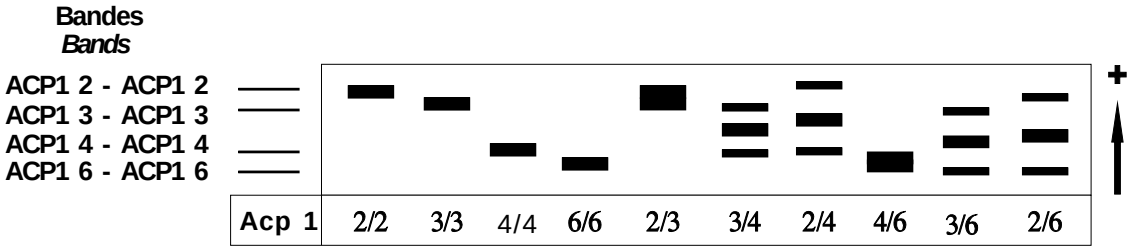
6.6 Recognition of the alleles encoding ACP

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.

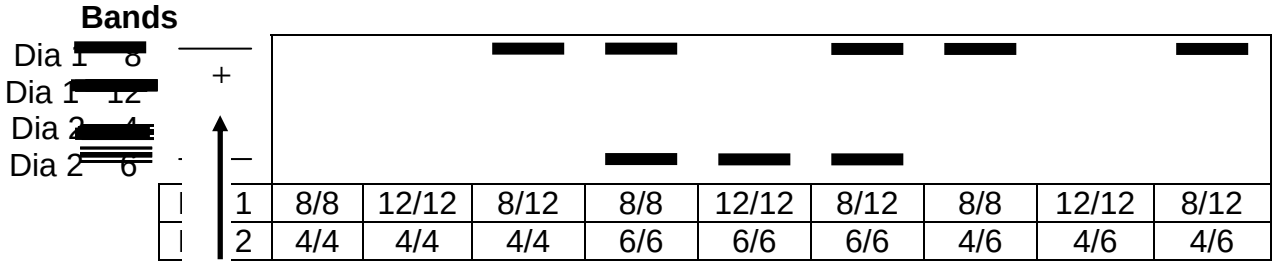
6.7 Recognition of the alleles encoding DIA

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



1	8/8	12/12	8/12	8/8	12/12	8/12	8/8	12/12	8/12
2	4/4	4/4	4/4	6/6	6/6	6/6	4/6	4/6	4/6

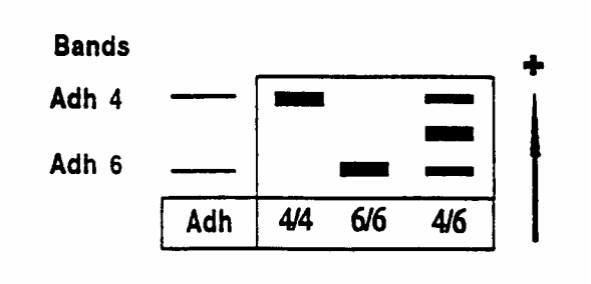
6.8 Recognition of the alleles encoding ADH

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



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Description of the example inbred lines

inbred lines	M	M	M	M	M	M	I	I	P	P	P	P
lignées endo-	d	d	d	m	d	d	d	d	g	g	g	g
games	h	h	h	m	h	h	h	h	d	d	m	m
Inzuchtlinien	1	2	3		4	5	1	2	1	2	1	2
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
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
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
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
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
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
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
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
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
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
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
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

Distinctness table between different levels of expression of characteristics 47.1.and 47.2

This table takes into account the fact that the levels of expression of some alleles can't be clearly identified when heterogynous genotypes are present

PGM1				9/9	9/9	9/9	9/9	9/9	9/9	16/16	9/9	9/9	9/9	9/9	16/16	16/16	16/16	16/16	5/5
	PGM2			1/1	1/3	3/3	3/4	4/4	1/4	4/4	8/8	3/8	4/8	1/8	1/1	1/3	3/3	8/8	3/3
		47.1		1		2		3		2	4				5		5	6	-
			47.2	1	1	1	1	1	1	1	2	2	2	3	4	4	4	5	-
				1	2	3	4	5	6	7(3)	8	8	8	9	10	10	10	11	12
9/9	1/1	1	1	no	?	yes	?	yes	?	yes	yes	yes	yes	yes	yes	yes	yes	yes	?
9/9	1/3		1	?	no	?	?	?	?	?	yes	yes	yes	yes	yes	yes	yes	yes	?
9/9	3/3	2	1	yes	?	no	?	yes	?	no	yes	yes	yes	yes	yes	yes	yes	yes	?
9/9	3/4		1	?	?	?	no	?	?	?	yes	yes	yes	yes	yes	yes	yes	yes	?
9/9	4/4	3	1	yes	?	yes	?	no	?	yes	yes	yes	yes	yes	yes	yes	yes	yes	?
9/9	1/4		1	?	?	?	?	?	no	?	yes	yes	yes	yes	yes	yes	yes	yes	?
16/16	4/4	2	1	yes	?	no	?	yes	?	no	yes	yes	yes	yes	yes	yes	yes	yes	?
9/9	8/8	4	2	yes	yes	yes	yes	yes	yes	yes	no	no	no	yes	yes	yes	yes	yes	?
9/9	3/8		2	yes	yes	yes	yes	yes	yes	yes	no	no	no	yes	yes	yes	yes	yes	?
9/9	4/8		2	yes	yes	yes	yes	yes	yes	yes	no	no	no	yes	yes	yes	yes	yes	?
9/9	1/8		3	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	?
16/16	1/1	5	4	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	no	no	no	yes	?
16/16	1/3		4	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	no	no	no	yes	?
16/16	3/3	5	4	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	no	no	no	yes	?
16/16	8/8	6	5	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	no	?
5/5	3/3	-	-	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	no

Distinctness table between different levels of expression of characteristics 49.1.and 4

This table takes into account the fact that the levels of expression of some alleles can't be clearly identif
when heterogynous genotypes are present

ACP1			2/2	2/3	3/3	4/6	4/4	6/6	2/4	2/6	3/4	3/6
	49.1		1		2		3	4				
		49.2	1	1	1	2	2	2	3	4	5	6
			1	2	3	4	5	6	7	8	9	10
2/2	1	1	?	?	yes	yes	yes	yes	yes	yes	yes	yes
2/3		1	?	?	?	yes	yes	yes	yes	yes	yes	yes
3/3	2	1	yes	?	?	yes	yes	yes	yes	yes	yes	yes
4/6		2	yes	yes	yes	?	?	?	yes	yes	yes	yes
4/4	3	2	yes	yes	yes	?	?	yes	yes	yes	yes	yes
6/6	4	2	yes	yes	yes	?	yes	no	yes	yes	yes	yes
2/4		3	yes	yes	yes	yes	yes	yes	no	yes	yes	yes
2/6		4	yes	yes	yes	yes	yes	yes	yes	no	yes	yes
3/4		5	yes	yes	yes	yes	yes	yes	yes	yes	no	yes
3/6		6	yes	yes	yes	yes	yes	yes	yes	yes	yes	no

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