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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

MAIZE *

UPOV Code: ZEAAA_MAY

Zea mays L.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

Alternative Names:*

Botanical name	*English*	*French*	*German*	*Spanish*
Zea mays L.	Maize, Corn	Maïs	Mais	Maíz

The purpose of these guidelines ("Test Guidelines") is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These Test Guidelines should be read in conjunction with the General Introduction and its associated TGP documents.

* These names were correct at the time of the introduction of these Test Guidelines but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Website (www.upov.int), for the latest information.]

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Zea mays* L. (excluding ornamental varieties).

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of seed.

2.3 The minimum quantity of plant material, to be supplied by the applicant, should be:

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

The seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority. In cases where the seed is to be stored, the germination capacity should be as high as possible and should be stated by the applicant.

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

3. Method of Examination

3.1 Number of Growing Cycles

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

3.2 Testing Place

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 “Examining Distinctness”.

3.3 Conditions for Conducting the Examination

The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

3.3.1 Stage of development for the assessment

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

3.3.2 Type of observation

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

MG: single measurement of a group of plants or parts of plants
MS: measurement of a number of individual plants or parts of plants
VG: visual assessment by a single observation of a group of plants or parts of plants

3.4 Test Design

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

3.5 Number of Plants / Parts of Plants to be Examined

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

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

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

3.6 Additional Tests

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

4. Assessment of Distinctness, Uniformity and Stability

4.1 Distinctness

4.1.1 General Recommendations

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

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

(i) description of parental lines according to the Test Guidelines;

(ii) check of the originality of the parental lines in comparison with the reference collection, based on the characteristics in Section 7 in order to screen the closest inbred lines;

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

(iv) assessment of the distinctness at the hybrid level of varieties with a similar formula.

4.1.2 Consistent Differences

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

4.1.3 Clear Differences

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

4.2 Uniformity

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

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

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

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

4.3 Stability

4.3.1 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability of inbred lines or open-pollinated varieties may be tested, either by growing a further generation, or by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the previous material supplied.

4.3.3 Where appropriate, or in cases of doubt, the stability of a hybrid variety may, in addition to an examination of the hybrid variety itself, also be assessed by examination of the uniformity and stability of its parent lines.

5. Grouping of Varieties and Organization of the Growing Trial

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

(a) Tassel: time of anthesis (characteristic 8)
(b) Tassel: anthocyanin coloration at base of glume (characteristic 9)
(c) Ear: anthocyanin coloration of silks (characteristic 16)
(d) Plant: length (characteristic 24)
(e) Ear: type of grain (characteristic 36)
(f) Excluding varieties with ear type of grain: sweet: Ear: color of dorsal side of grain (characteristic 40)
(g) Ear: anthocyanin coloration of glumes of cob (characteristic 42)

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

6. Introduction to the Table of Characteristics

6.1 Categories of Characteristics

6.1.1 Standard Test Guidelines Characteristics

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

6.1.2 Asterisked Characteristics

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

6.2 States of Expression and Corresponding Notes

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

6.3 Types of Expression

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

6.4 Example Varieties

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

6.5 Legend

(*) Asterisked characteristic – see Chapter 6.1.2

QL: Qualitative characteristic – see Chapter 6.3
QN: Quantitative characteristic – see Chapter 6.3
PQ: Pseudo-qualitative characteristic – see Chapter 6.3

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

MG, MS, VG: See Chapter 3.3.2

PC: Popcorn variety
SC: Sweet corn variety

(a)-(e) See explanations on the Table of Characteristics in Chapter 8.1
(+) See explanations on the Table of Characteristics in Chapter 8.2
14-93 See explanations on the Table of Characteristics in Chapter 8.2 (Decimal Code for the Growth Stages)

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

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

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

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

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
10. **(+)**	**65-69 (S) 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**	**Panícula: pigmentación antociánica de las glumas, con exclusión de la base**		
QN	**(b)**	absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Empire (SC), F259	1
		weak	faible	gering	débil	F2, Royalty (SC)	3
		medium	moyenne	mittel	media	Centurion (SC), WD36	5
		strong	forte	stark	fuerte	W79A	7
		very strong	très forte	sehr stark	muy fuerte		9
11. **(+)**	**VG (S)**	**Tassel: anthocyanin coloration of anthers**	**Panicule: pigmentation anthocyanique des anthères**	**Rispe: Anthocyanfärbung der Antheren**	**Panícula: pigmentación antociánica de las anteras**		
QN	**(b)**	absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	A654, Empire (SC)	1
		weak	faible	gering	débil	F2, Royalty (SC)	3
		medium	moyenne	mittel	media	Centurion (SC), W182E	5
		strong	forte	stark	fuerte		7
		very strong	très forte	sehr stark	muy fuerte		9
12. **(*)** **(+)**	**65-69 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**	**Panícula: ángulo entre el eje central y las ramas laterales**		
QN	**(c)**	very small	très petit	sehr klein	muy pequeño		1
		small	petit	klein	pequeño	F492	3
		medium	moyen	mittel	medio	EP1, Mv. Aranyos (SC)	5
		large	grand	groß	grande	Bonus (SC), F186	7
		very large	très grand	sehr groß	muy grande		9

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

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

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

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

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
23.	**71-75 MS**	**Tassel: length of lateral branch**	**Panicule: longueur du rameau**	**Rispe: Länge der Seitenäste**	**Panícula: longitud de la rama lateral lateral**		
QN	**(c)**	very short	très court	sehr kurz	muy corta		1
		short	court	kurz	corta	EP1	3
		medium	moyen	mittel	media	A632	5
		long	long	lang	larga	F492	7
		very long	très long	sehr lang	muy larga		9
24.1 (*) (+)	**MS 75-85**	**<u>Only inbred lines:</u> Plant: length**	**<u>Seulement pour les lignées endogames</u>: Plante: longueur**	**<u>Nur Inzuchtlinien</u>: Pflanze: Länge**	**<u>Sólo líneas endógamas:</u> Planta: longitud**		
QN		very short	très courte	sehr kurz	muy corta	F7	1
		very short to short	très courte à courte	sehr kurz bis kurz	muy corta a corta		2
		short	courte	kurz	corta	W117	3
		short to medium	courte à moyenne	kurz bis mittel	corta a media		4
		medium	moyenne	mittel	media	F244	5
		medium to long	moyenne à longue	mittel bis lang	media a larga		6
		long	longue	lang	larga	WD36	7
		long to very long	longue à très longue	lang bis sehr lang	larga a muy larga		8
		very long	très longue	sehr lang	muy larga		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
24.2 (*) (+)	**MS 75-85**	**Only hybrids and open-pollinated varieties: Plant: length**	**Seulement pour les hybrides et les variétés à fécondation libre: Plante: longueur**	**Nur Hybriden und freiabblühende Sorten: Pflanze: Länge**	**Sólo híbridos y variedades de polinización libre: Planta: longitud**		
QN		very short	très courte	sehr kurz	muy corta		1
		very short to short	très courte à courte	sehr kurz bis kurz	muy corta a corta		2
		short	courte	kurz	corta	PR39D23, Spirit (SC)	3
		short to medium	courte à moyenne	kurz bis mittel	corta a media		4
		medium	moyenne	mittel	media	PR37Y12, Puma (SC)	5
		medium to long	moyenne à longue	mittel bis lang	media a larga		6
		long	longue	lang	larga	DKC5166, Royalty (SC)	7
		long to very long	longue à très longue	lang bis sehr lang	larga a muy larga		8
		very long	très longue	sehr lang	muy larga	Enterprise (SC)	9
25. (+)	**75-85 MG**	**Plant: ratio height of insertion of peduncle of upper ear to plant length**	**Plante: hauteur d'insertion du pédoncule de l'épi le plus haut par rapport à la longueur de la plante**	**Pflanze: Verhältnis der Ansatzhöhe des Kolbenstiels des obersten Kolbens zur Pflanzenlänge**	**Planta: relación entre la altura de inserción del pedúnculo de la mazorca más alta y la longitud de la planta**		
QN		very small	très petit	sehr klein	muy pequeña	Gyöngymazsola (SC)	1
		small	petit	klein	pequeña	F816, Spirit (SC)	3
		medium	moyen	mittel	media	F252, Royalty (SC)	5
		large	grand	groß	grande	F481	7
		very large	très grand	sehr groß	muy grande		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
26.	**75-85 MS**	**Leaf: width of blade**	**Feuille: largeur du limbe**	**Blatt: Breite der Spreite**	**Hoja: anchura del limbo**		
QN	**(a)**	very narrow	très étroit	sehr schmal	muy estrecho		1
		narrow	étroit	schmal	estrecho	Champ (SC), F16	3
		medium	moyen	mittel	medio	Empire (SC), F244	5
		wide	large	breit	ancho	Centurion (SC), F481	7
		very wide	très large	sehr breit	muy ancho		9
27.	**75-85 VG**	**Peduncle: length**	**Pédoncule: longueur**	**Kolbenstiel: Länge**	**Pedúnculo: longitud**		
QN		very short	très court	sehr kurz	muy corto		1
		short	court	kurz	corto	Centurion (SC), F259	3
		medium	moyen	mittel	medio	A654, Jubilee (SC)	5
		long	long	lang	largo	F107	7
		very long	très long	sehr lang	muy largo		9
28. (*) (+)	**92-93 sweet -corn 75-79 MS**	**Ear: length**	**Épi: longueur**	**Kolben: Länge**	**Mazorca: longitud**		
QN		very short	très court	sehr kurz	muy corta		1
		short	court	kurz	corta	F2	3
		medium	moyen	mittel	media	A654, Spirit (SC)	5
		long	long	lang	larga	Empire (SC), MO17	7
		very long	très long	sehr lang	muy larga		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
29.	**92-93 sweet -corn 75-79 MS**	**Ear: diameter (in middle)**	**Épi: diamètre (au milieu)**	**Kolben: Durch-messer (in der Mitte)**	**Mazorca: diámetro (en el medio)**		
QN		very small	très petit	sehr klein	muy pequeño		1
		small	petit	klein	pequeño	F7	3
		medium	moyen	mittel	medio	W117	5
		large	grand	groß	grande	Centurion (SC), F481	7
		very large	très grand	sehr groß	muy grande	Empire (SC)	9
30. (+)	**92-93 sweet -corn 75-79 VG**	**Ear: shape**	**Epi: forme**	**Kolben: Form**	**Mazorca: forma**		
QN		conical	conique	konisch	cónica	F16, Wombat (SC)	1
		conico-cylindrical	cylindro-conique	konisch-zylindrisch	cilindrocónica	Centurion (SC), F816	2
		cylindrical	cylindrique	zylindrisch	cilíndrica	F66, GH2547 (SC)	3
31.	**92-93 sweet -corn 75-93 MS**	**Ear: number of rows of grain**	**Épi: nombre de rangs**	**Kolben: Anzahl der Kornreihen**	**Mazorca: número de hileras de granos**		
QN		very few	très petit	sehr gering	muy bajo		1
		few	petit	gering	bajo	F257	3
		medium	moyen	mittel	medio	Dessert 73 (SC), F16	5
		many	grand	groß	alto	B73, Bonus (SC)	7
		very many	très grand	sehr groß	muy alto		9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
32.	**75-79 (S) VG**	**Only varieties with ear type of grain: sweet, pop or waxy: Ear: number of colors of grains**	**Seulement pour les variétés avec type de grain de l'épi: doux, à éclater ou cireux: Épi: nombre de couleurs de grains**	**Nur Sorten mit Kolben: Korntyp: Zuckermais, Popcorn oder Wachsmais: Kolben: Anzahl Farben der Körner**	**Sólo variedades con mazorca con tipo de grano: dulce, palomero o ceroso: Mazorca: número de colores de los granos**		
QL	**(e)**	one	une	eine	uno	Jubilee (SC)	1
		two	deux	zwei	dos	Eolrukchal-ilho, Serendipity (SC)	2
33. (*)	**75-79 VG**	**Only varieties with ear type of grain: sweet: Grain: intensity of yellow color**	**Seulement pour les variétés avec le type de grain: doux: Grain: intensité de la couleur jaune**	**Nur Sorten mit Kolben: Korntyp: Zuckermais: Korn: Intensität der Gelbfärbung**	**Sólo variedades con mazorca con tipo de grano: dulce: Grano: intensidad del color amarillo**		
QN	**(e)**	light	claire	hell	claro	Gyöngymazsola (SC)	3
		medium	moyenne	mittel	medio	Royalty (SC)	5
		dark	foncée	dunkel	oscuro	Kokanee (SC)	7
34.	**75-79 VG**	**Only varieties with ear type of grain: sweet: Grain: length**	**Seulement pour les variétés avec le type de grain de l'épi: doux: Grain: longueur**	**Nur Sorten mit Kolben: Korntyp: Zuckermais: Korn: Länge**	**Sólo variedades con mazorca con tipo de grano: dulce: Grano: longitud**		
QN	**(d)**	short	court	kurz	corto		3
		medium	moyen	mittel	medio	Boston (SC)	5
		long	long	lang	largo	GH5704 (SC)	7
35. (+)	**75-79 VG**	**Only varieties with ear type of grain: sweet: Grain: width**	**Seulement pour les variétés avec le type de grain de l'épi: doux: Grain: largeur**	**Nur Sorten mit Kolben: Korntyp: Zuckermais: Korn: Breite**	**Sólo variedades con mazorca con tipo de grano: dulce: Grano: anchura**		
QN	**(d)**	narrow	étroit	schmal	estrecho	Bonus (SC)	3
		medium	moyen	mittel	medio	Jubilee (SC)	5
		broad	large	breit	ancho	Mv. Aranyos (SC)	7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
36. (*) (+)	**92 (S) VG**	**Ear: type of grain**	**Épi: type de grain**	**Kolben: Korntyp**	**Mazorca: tipo de grano**		
QL	**(d)**	flint	corné	Hartmais	córneo	F2	1
	(e)	flint-like	corné à corné-denté	hartmaisähnlich	córneo a córneo-dentado	F252	2
		intermediate	corné-denté	Zwischentyp	córneo-dentado	F107	3
		dent-like	corné-denté à denté	zahnmaisähnlich	córneo-dentado a dentado	A654	4
		dent	denté	Zahnmais	dentado	W182E	5
		sweet	sucré	Zuckermais	dulce	Jubilee (SC)	6
		pop	à éclater	Popcorn	palomero	Iowa Pop (PC)	7
		waxy	cireux	Wachsmais	ceroso		8
		flour	farineux	Mehlmais	harinoso		9
37. (*) (+)	**92 VG**	**<u>Only varieties with ear type of grain: sweet:</u> Ear: shrinkage of top of grain**	**<u>Seulement variétés avec type de grain de l'épi: doux</u>: Épi: rétrécissement de la partie supérieure du grain**	**<u>Nur Sorten mit Kolben: Korntyp: Zuckermais</u>: Kolben: Schrumpfung Kornkrone**	**<u>Sólo variedades con mazorca con tipo de grano: dulce:</u> Mazorca: contracción del extremo superior del grano**		
QN	**(d)**	weak	faible	gering	débil	Zarja (SC)	1
	(e)	medium	moyenne	mittel	media	Merkur (SC)	3
		strong	forte	stark	fuerte	Dessert 73 (SC)	5

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
38. (*) (+)	**92-93 (S) VG**	**Ear: main color of top of grain**	**Épi: couleur principale du sommet du grain**	**Kolben: Hauptfarbe der Kornkrone**	**Mazorca: color principal del extremo superior del grano**		
PQ	**(d)**	white	blanc	weiß	blanco	A188, Pure white (SC), Snowbelle (SC), Sugar Purple (SC)	1
	(e)	yellowish white	blanc jaunâtre	gelblich weiß	blanco amarillento		2
		yellow	jaune	gelb	amarillo	F259, SSActivate (SC)	3
		yellow orange	jaune orangé	gelborange	amarillo anaranjado	F2, Gyöngymazsola (SC)	4
		orange	orange	orange	naranja	F257, GH 2547 (SC)	5
		red orange	rouge orangé	rotorange	naranja rojizo	Dynasty (SC)	6
		red	rouge	rot	rojo		7
		purple	pourpre	purpur	púrpura	Dynared (SC), Wild Violet (SC)	8
		brownish	brunâtre	bräunlich	amarronado	Zenith (SC)	9
		bluish black	noir bleuâtre	bläulichschwarz	negro azulado	Miheukchal	10

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
39. (*) (+)	**VG 92-93**	**Only varieties with ear type of grain: sweet, pop or waxy: Ear: secondary color of top of grain**	**Seulement pour les variétés avec type de grain de l'épi: doux, à éclater ou cireux: Épi: couleur secondaire du sommet du grain**	**Nur Sorten mit Kolben: Korntyp: Zuckermais, Popcorn oder Wachsmais: Kolben: Sekundärfarbe der Kornkrone**	**Sólo variedades con mazorca con tipo de grano: dulce, palomero o ceroso: Mazorca: color secundario del extremo superior del grano**		
PQ		white	blanc	weiß	blanco	SSActivate (SC), Wild Violet (SC)	1
		yellowish white	blanc jaunâtre	gelblich weiß	blanco amarillento		2
		yellow	jaune	gelb	amarillo		3
		yellow orange	jaune orangé	gelborange	amarillo anaranjado		4
		orange	orange	orange	naranja		5
		red orange	rouge orangé	rotorange	naranja rojizo		6
		red	rouge	rot	rojo		7
		purple	pourpre	purpur	púrpura	Sugar Purple (SC)	8
		brownish	brunâtre	bräunlich	amarronado		9
		bluish black	noir bleuâtre	bläulichschwarz	negro azulado		10

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
40. (*)	**92-93 (S) VG**	**Excluding varieties with ear type of grain: sweet: Ear: color of dorsal side of grain**	**À l'exclusion des variétés avec type de grain de l'épi: doux: Épi: couleur de la face dorsale du grain**	**Ohne Sorten mit Kolben: Korntyp: Zuckermais: Kolben: Farbe der Kornrückseite**	**Excepto variedades con mazorca con tipo de grano: dulce: Mazorca: color del lado dorsal del grano**		
PQ	**(d)**	white	blanc	weiß	blanco	F481	1
	(e)	yellowish white	blanc jaunâtre	gelblich weiß	blanco amarillento	A188	2
		yellow	jaune	gelb	amarillo		3
		yellow orange	jaune orangé	gelborange	amarillo anaranjado	F66	4
		orange	orange	orange	naranja	EP1	5
		red orange	rouge orangé	rotorange	naranja rojizo		6
		red	rouge	rot	rojo		7
		purple	pourpre	purpurn	púrpura		8
		brownish	brunâtre	bräunlich	amarronado		9
		bluish black	noir bleuâtre	bläulichschwarz	negro azulado		10
41. (+)	**93 VG**	**Only varieties with ear type of grain: pop: Type of popped grain**	**Seulement pour les variétés avec le type de grain de l'épi: à éclater: Type de grain éclaté**	**Nur Sorten mit Kolben: Korntyp: Popcorn: Typ des gepufften Korns**	**Sólo variedades con mazorca con tipo de grano: palomero: Tipo del grano reventado**		
QN		butterfly	à ailettes	Schmetterlingtyp	palomita	Robust 97461 (PC)	1
		intermediate	intermédiaire	Zwischentyp	intermedio		2
		globular	globuleux	Kugeltyp	globular	Robust 90252 (PC)	3

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
42. (*) (+)	**93 (S) VG**	**Ear: anthocyanin coloration of glumes of cob**	**Épi: pigmentation anthocyanique des glumes de la rafle**	**Kolben: Anthocyan-färbung der Spelzen der Spindel**	**Mazorca: pigmentación antociánica de las glumas del zuro**		
QN		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	F2, F257	1
		weak	faible	gering	débil	F252	3
		medium	moyenne	mittel	media	W117	5
		strong	forte	stark	fuerte	A632	7
		very strong	très forte	sehr stark	muy fuerte		9

8. Explanations on the Table of Characteristics

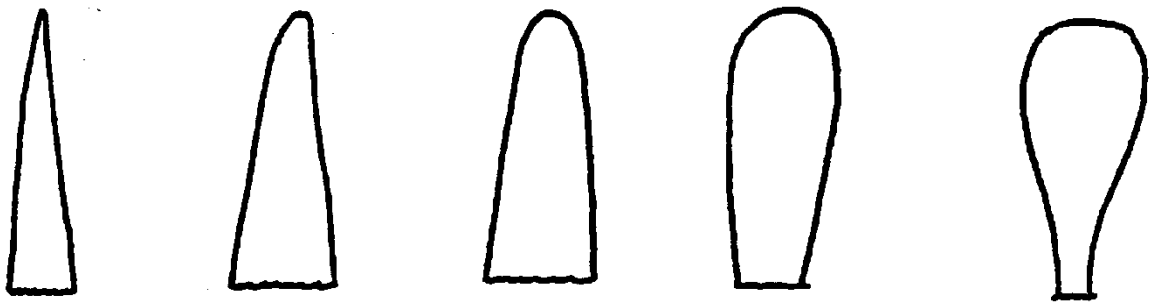
8.1 Explanations covering several characteristics

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

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

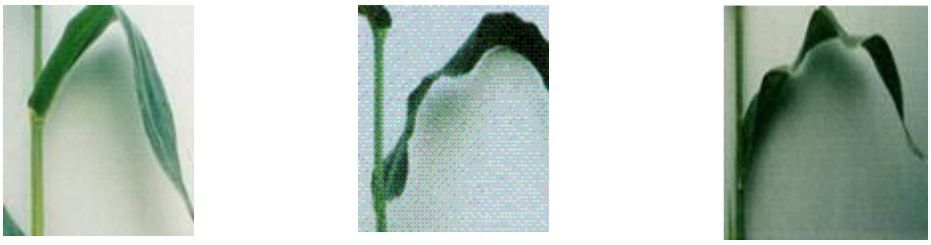
8.2 Explanations for individual characteristics

Ad. 2: First leaf: shape of apex



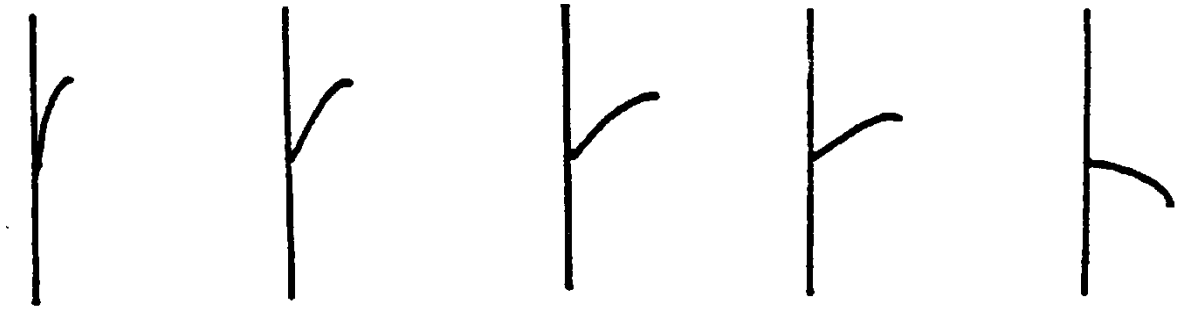
1	2	3	4	5
pointed	pointed to rounded	rounded	rounded to spatulate	spatulate

Ad. 4: Leaf: undulation of margin of blade



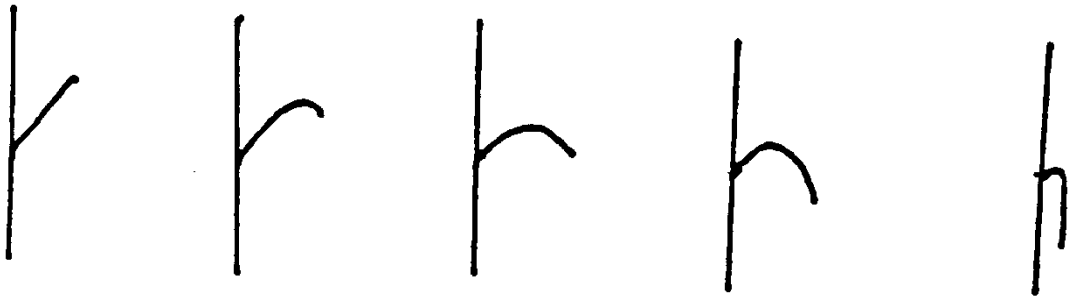
1	2	3
absent or very weak	intermediate	strong

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	small	medium	large	very large
(< 5°)	(± 25°)	(± 50°)	(± 75°)	(> 90°)

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

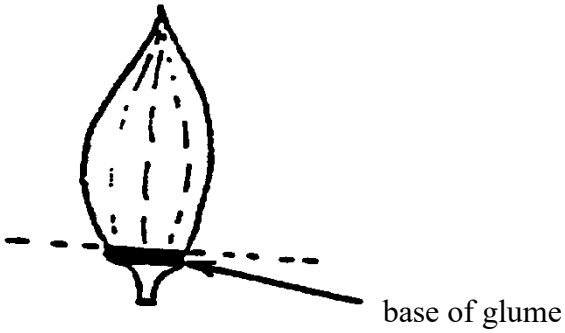


1	3	5	7	9
absent or very slightly recurved	slightly recurved	moderately recurved	strongly recurved	very strongly recurved

Ad. 8: Tassel: time of anthesis

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

Ad. 9: Tassel: anthocyanin coloration at base of glume
Ad. 10: Tassel: anthocyanin coloration of glumes excluding base



Ad. 11: Tassel: anthocyanin coloration of anthers

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

Ad. 15: Ear: time of silk emergence

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

Ad. 17: Stem: anthocyanin coloration of brace roots

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

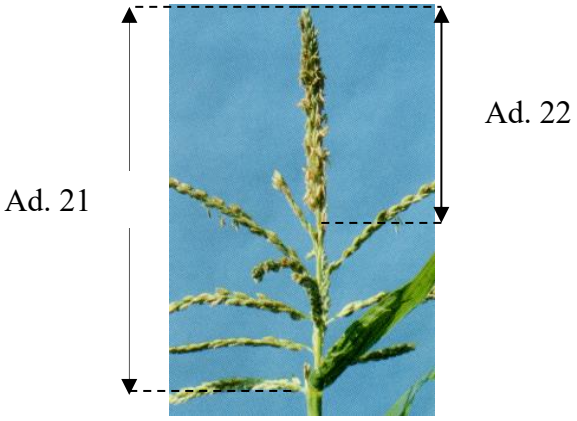
Ad. 19: Leaf: anthocyanin coloration of sheath

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

Ad. 20: Stem: anthocyanin coloration of internodes

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

Ad. 21: Tassel: length of main axis above lowest lateral branch
Ad. 22: Tassel: length of main axis above highest lateral branch

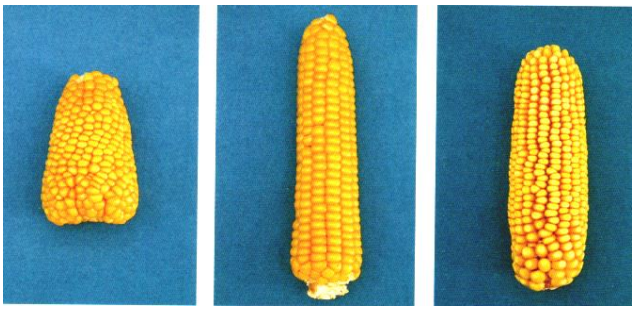


Ad. 24.1: Only inbred lines: Plant: length
Ad. 24.2: Only hybrids and open-pollinated varieties: Plant: length
Ad. 25: Plant: ratio height of insertion of peduncle of upper ear to plant length

The plant length should be observed including the tassel.

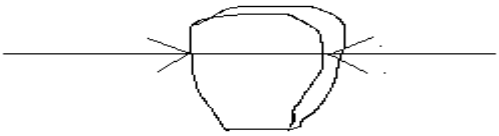
Ad. 28: Ear: length

Ad. 30: Ear: shape



1 conical | 2 conico-cylindrical | 3 cylindrical

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



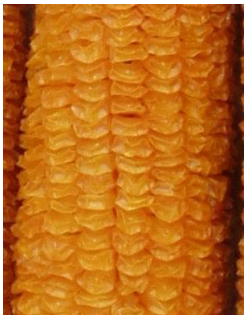
Ad. 36: Ear: type of grain

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

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

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

1
weak



3
medium

5
strong

Ad. 38: Ear: main color of top of grain
Ad. 39: Only varieties with ear type of grain: sweet, pop or waxy: Ear: secondary color of top of grain

The color with the largest surface area is the main color; the one with the second largest area is the secondary color.

In cases where the areas of the main and secondary color are too similar to reliably decide which color has the largest area, the darker color is considered to be the main color.

Note: caution should be taken if no full isolation of varieties with different colors in the trial is practiced. Cross pollination from other samples can in that case cause small amounts of grain with a different color which do not belong to the variety. Hence the variety is not a variety with two colors (bicolor variety) of itself.

Ad. 41: Only varieties with ear type of grain: pop: Type of popped grain

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

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

1
butterfly

3
globular

Ad. 42: Ear: anthocyanin coloration of glumes of cob

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

*8.3 Decimal Code for the Growth Stages**

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

CODE	GENERAL DESCRIPTION		DESCRIPTION
	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

---

* Extracted from J.C. Zadoks, T.T. Chang and C.F. Konzak except (1), Decimal Code for the Growth States of Cereals, EUCARPIA Bulletin No. 7, 1974, pp. 42-52. The French translation has been kindly furnished by Mrs. R. Cassini, Mr. R. Cassini and Mr. R. Marie. The German translation has been kindly furnished by Mr. A.O. Klomp and Mrs. I. Volk.

* Extrait de J.C. Zadoks, T.T. Chang et C.F. Konzak excepté (1), Decimal Code for the Growth States of Cereals, EUCARPIA Bulletin No. 7, 1974, pp. 42-52. La traduction française a été aimablement fournie par Mme R. Cassini, M. R. Cassini et M. R. Marie. La traduction allemande a été aimablement fournie par M. A.O. Klomp et Mme I. Volk.

* Auszug von J.C. Zadoks, T.T. Chang und C.F. Konzak außer (1), Decimal Code for the Growth States of Cereals, EUCARPIA Bulletin No. 7, 1974, pp. 42-52. Die französische Übersetzung wurde freundlicherweise von Frau R. Cassini, Herrn R. Cassini und Herrn R. Marie überlassen. Die deutsche Uebersetzung wurde freundlicherweise von Herrn A.O. Klomp und Frau I. Volk überlassen.

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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 (provide details) [ ]

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]
(please state parent varieties)

(b) partially known cross [ ]
(please state known parent variety(ies))

(c) unknown cross [ ]

4.1.2 Mutation [ ]
(please state parent variety)

4.1.3 Discovery and development [ ]
(please state where and when discovered and how developed)

4.1.4 Other [ ]
(please provide details)

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

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

4.2 Method of propagating the variety

##4.2.1 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.

(a) Single Hybrid

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

(b) Three-Way Hybrid

single hybrid (below) used as female parent x (….....…)
male parent line
or (…….....…) x single hybrid (below) used as male parent
female parent line

(….....…) x (….....…)
female parent line male parent line
single hybrid

(c) Double Hybrid

(….....…) x (….....…)
female parent line male parent line
single hybrid used as female parent

(….....…) x (….....…)
female parent line 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:

(i) any male sterile female parent lines

……

(ii) maintenance system of male sterile female parent lines

Authorities may choose to request this information

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

4.2.2 Open-pollinated variety (please provide details)

……

4.2.3 Other (please provide details)

……

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

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

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

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

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

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

	Characteristics	Example Varieties	Note
5.7 (16)	**Ear: anthocyanin coloration of silks**		
	absent or very weak	Bonus (SC), F7, F195	1[ ]
	very weak to weak		2[ ]
	weak	El Toro (SC), F257	3[ ]
	weak to medium		4[ ]
	medium	F244, Gyöngymazsola (SC)	5[ ]
	medium to strong		6[ ]
	strong	W401	7[ ]
	strong to very strong		8[ ]
	very strong		9[ ]
5.8 i (24.1)	**<u>Only inbred lines:</u> Plant: length**		
	very short	F7	1[ ]
	very short to short		2[ ]
	short	W117	3[ ]
	short to medium		4[ ]
	medium	F244	5[ ]
	medium to long		6[ ]
	long	WD36	7[ ]
	long to very long		8[ ]
	very long		9[ ]

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

	Characteristics	Example Varieties	Note
5.8 ii (24.2)	**<u>Only hybrids and open-pollinated varieties</u>: Plant: length**		
	very short		1[ ]
	very short to short		2[ ]
	short	PR39D23, Spirit (SC)	3[ ]
	short to medium		4[ ]
	medium	PR37Y12, Puma (SC)	5[ ]
	medium to long		6[ ]
	long	DKC5166, Royalty (SC)	7[ ]
	long to very long		8[ ]
	very long	Enterprise (SC)	9[ ]
5.9 (28)	**Ear: length**		
	very short		1[ ]
	very short to short		2[ ]
	short	F2	3[ ]
	short to medium		4[ ]
	medium	A654, Spirit (SC)	5[ ]
	medium to long		6[ ]
	long	Empire (SC), MO17	7[ ]
	long to very long		8[ ]
	very long		9[ ]

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

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

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

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

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

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

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

	Characteristics	Example Varieties	Note
5.18 (38)	**Ear: main color of top of grain**		
	white	A188, Pure white (SC), Snowbelle (SC), Sugar Purple (SC)	1 [ ]
	yellowish white		2 [ ]
	yellow	F259, SSActivate (SC)	3 [ ]
	yellow orange	F2, Gyöngymazsola (SC)	4 [ ]
	orange	F257, GH 2547 (SC)	5 [ ]
	red orange	Dynasty (SC)	6 [ ]
	red		7 [ ]
	purple	Dynared (SC), Wild Violet (SC)	8 [ ]
	brownish	Zenith (SC)	9 [ ]
	bluish black	Miheukchal	10 [ ]
5.19 (39)	**<u>Only varieties with ear type of grain: sweet, pop or waxy:</u> Ear: secondary color of top of grain**		
	white	SSActivate (SC), Wild Violet (SC)	1 [ ]
	yellowish white		2 [ ]
	yellow		3 [ ]
	yellow orange		4 [ ]
	orange		5 [ ]
	red orange		6 [ ]
	red		7 [ ]
	purple	Sugar Purple (SC)	8 [ ]
	brownish		9 [ ]
	bluish black		10 [ ]

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

	Characteristics	Example Varieties	Note
5.20 (40)	**<u>Excluding varieties with ear type of grain: sweet</u>: Ear: color of dorsal side of grain**		
	white	F481	1 [ ]
	yellowish white	A188	2 [ ]
	yellow		3 [ ]
	yellow orange	F66	4 [ ]
	orange	EP1	5 [ ]
	red orange		6 [ ]
	red		7 [ ]
	purple		8 [ ]
	brownish		9 [ ]
	bluish black		10 [ ]
5.21 (42)	**Ear: anthocyanin coloration of glumes of cob**		
	absent or very weak	F2, F257	1 [ ]
	very weak to weak		2 [ ]
	weak	F252	3 [ ]
	weak to medium		4 [ ]
	medium	W117	5 [ ]
	medium to strong		6 [ ]
	strong	A632	7 [ ]
	strong to very strong		8 [ ]
	very strong		9 [ ]

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

6. Similar varieties and differences from these varieties

Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the **similar** variety(ies)	Describe the expression of the characteristic(s) for **your** candidate variety
Example	*Ear: time of silk emergence*	*early*	*early to medium*

Comments:

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

[#]7. Additional information which may help in the examination of the variety

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

Yes [ ] No [ ]

(If yes, please provide details)

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

Yes [ ] No [ ]

(If yes, please provide details)

7.3 Other information

Sweetcorn varieties only: type

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

Other information

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

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”.

……

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

Applicant’s name

Signature Date

[Annex follows]

ANNEX

Additional Useful Explanations

TABLE OF CONTENTS PAGE

Part I. Introduction 52
Part II. Characteristics derived by Isozyme Polymorphism 52
Part III. Description of the SGE Method for the Analysis of Isozymes from *Zea mays* L. 56

Part I

Introduction

The following Annex contains a list of characteristics based on isozyme markers revealed by electrophoresis and a description of the method to be used. UPOV decided to place these characteristics in an Annex to the Test Guidelines, thereby creating a special category of characteristic, because the majority of the UPOV member States is of the view that it is not possible to establish distinctness solely on the basis of a difference found in a characteristic based on isozyme markers revealed by electrophoresis. Such characteristics should therefore only be used as a complement to other differences in morphological or physiological characteristics. UPOV reconfirms that these characteristics are considered useful but that they might not be sufficient on their own to establish distinctness. They should not be used as a routine characteristic but at the request or with the agreement of the applicant of the candidate variety.

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

Part II

Characteristics derived by Isozyme Polymorphism

Characteristics			Examples	Note
42.	***Allele expression at locus Mdh 1***	Genotype 1/1	F252	1
		Genotype 0.5/0.5	R3126	
QL		Genotype 0.5/1	KW 5361 xKW 5454	
		Genotype 1/6 in interaction with allele 6 of Mdh 2	Tau	
		Genotype 0.5/6 in interaction with allele 6 of Mdh 2	Clarica	
		Genotype 6/6	A239	2
		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	

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

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

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

Part III

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

1. Number of coleoptiles per test

- for checking formula: at least 20 coleoptiles of each inbred line
  2 coleoptiles of single-cross hybrids
  6 coleoptiles of three-way cross hybrids

- for distinctness, uniformity and stability test: at least 20 coleoptiles for inbred lines, hybrids and open-pollinated varieties.

2. Apparatus and equipment

Any suitable horizontal electrophoresis system can be used, provided that the gels can be kept at 4°C. A gel thickness of 10 mm is recommended. The power supply used should be capable of delivering constant voltage output.

3. Chemicals

All chemicals should be of 'Analytical Reagent' grade or better.

3.1 Chemicals for enzyme extraction

L-Ascorbic acid
L-Ascorbic acid Na salt
Sucrose

3.2 Chemicals for electrophoresis

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

3.3 Chemicals for staining enzymes

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

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

4. Solutions

4.1 Extraction solution

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

4.2 Electrophoresis buffers

4.2.1 Buffers for SGE pH 6.5

4.2.1.1 Stock solution : 0.364 M L-histidine-citrate
50.44 g L-histidine
8.20 g Citric acid monohydrate
made up to 1 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 isozymes

6.1 Recognition of the alleles encoding MDH

6.1.1 Genetic interpretation of the zymogrammes

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

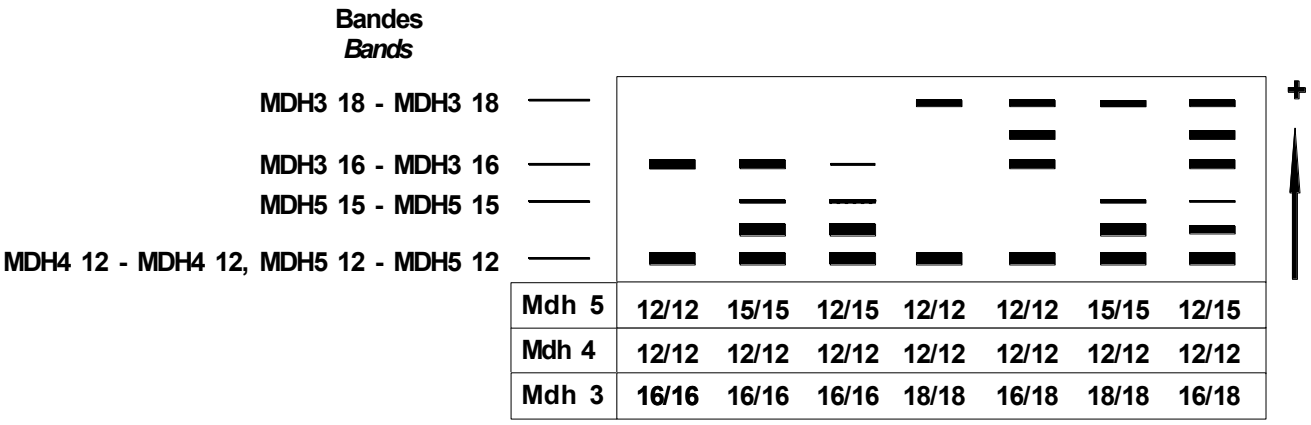
- Alleles 0.5 and 1 from Mdh1 are difficult to discriminate from each other. Therefore, they are scored as identical (note 1). The same is true for alleles 3 and 3.5 from Mdh2 which are scored together (note 1)
- There are interactions between the products of the genes (polypeptide subunits) on the one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

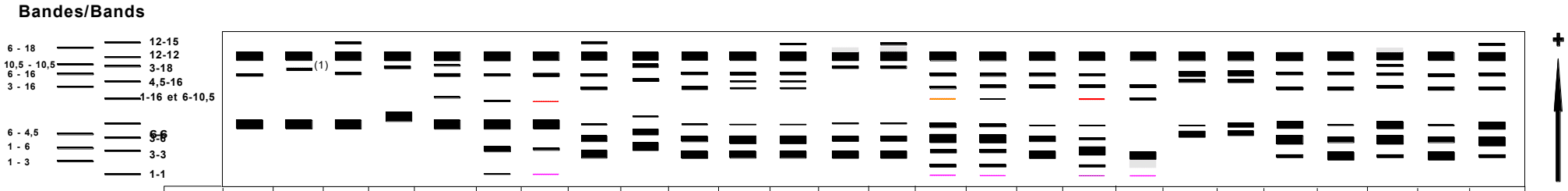
For the recognition of the alleles at the loci Mdhl, Mdh2 and Mdh4 the SGE at pH 6.5 should be used. For the recognition of the alleles at the loci Mdh3 and Mdh5, a second electrophoresis system should be used : SGE at pH 5.0.

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



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

6.2 Recognition of the alleles encoding IDH

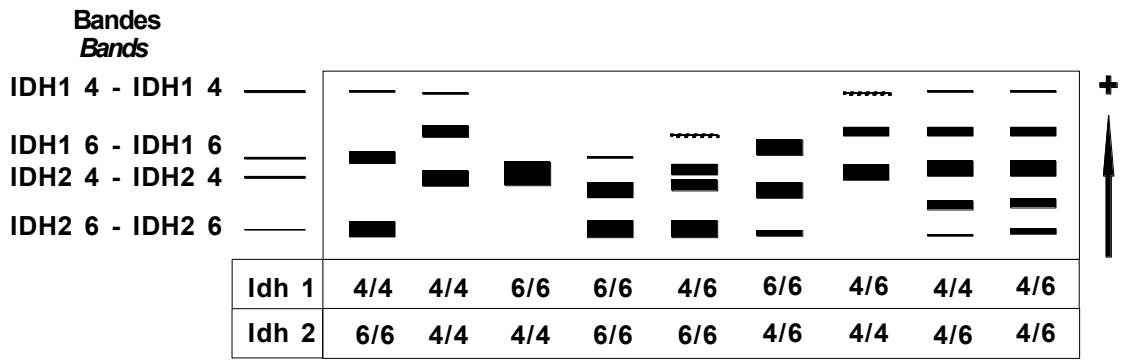
6.2.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal location	Locus	Alleles	
Isocitrate		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

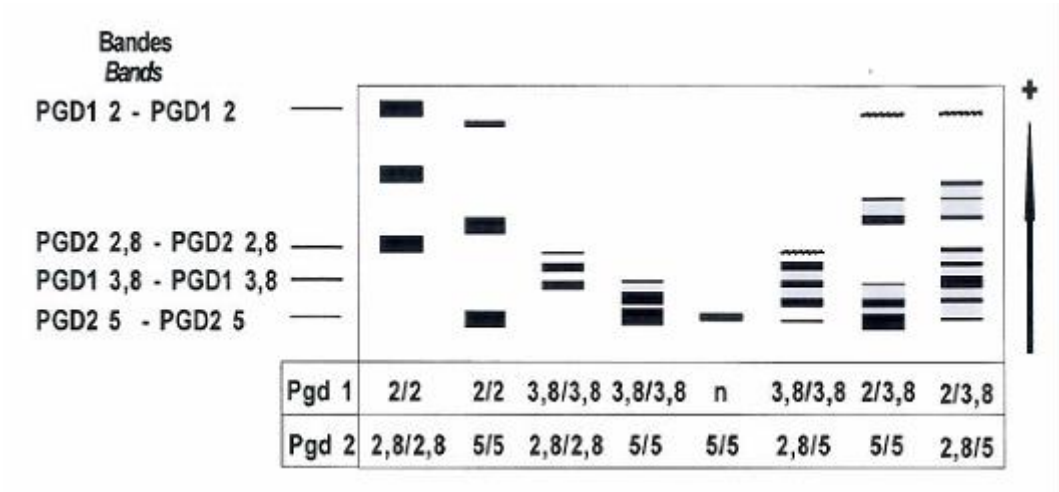
6.3.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Chromosomal 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.

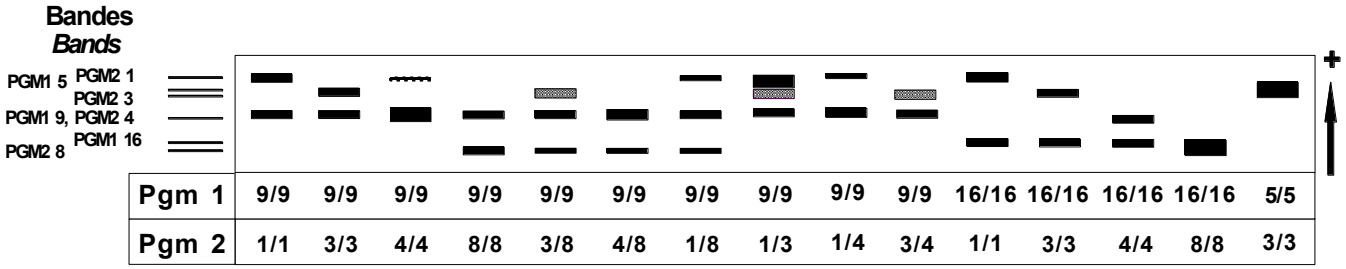
6.4 Recognition of the alleles encoding PGM

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



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

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

Combinations indicated with "+" can be clearly separated. In general, combinations indicated with "-" cannot be separated.
The notes within grey zones should not be used without knowledge of the parent formula.

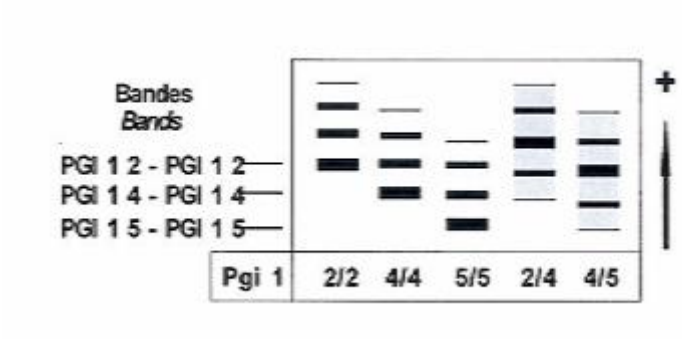
6.5 Recognition of the alleles encoding PGI

6.5.1 Genetic interpretation of the zymogrammes

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

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

6.5.2 Schematization of the zymogrammes



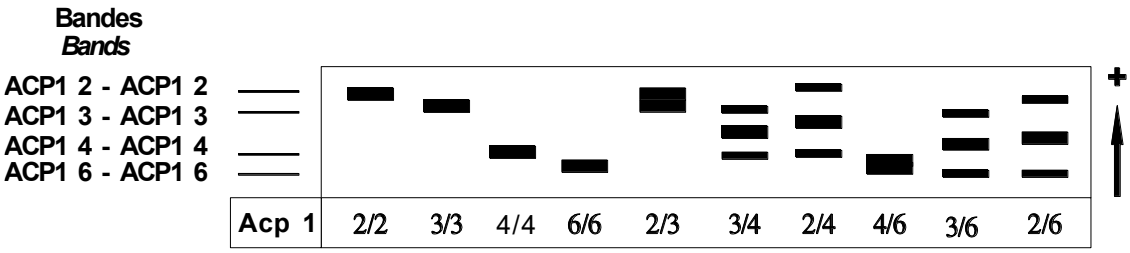
6.6 Recognition of the alleles encoding ACP

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.

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

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

Combinations indicated with "+" can be clearly separated. In general, combinations indicated with "-" cannot be separated.
The notes within grey zones should not be used without knowledge of the parent formula.

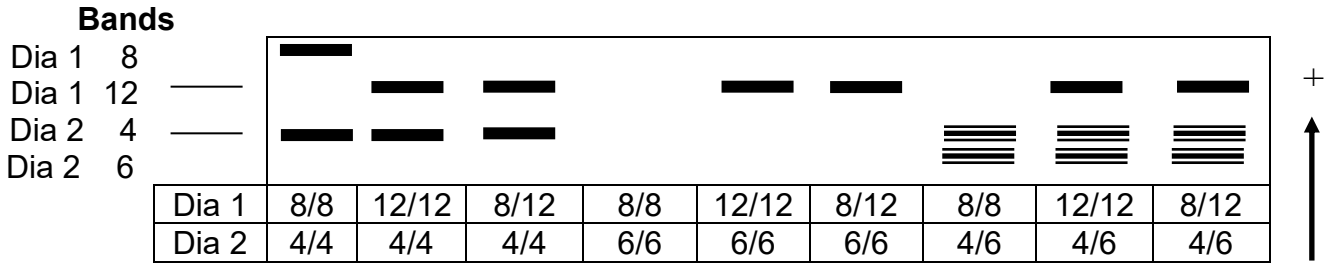
6.7 Recognition of the alleles encoding DIA

6.7.1 Genetic interpretation of the zymogrammes

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

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

6.7.2 Schematization of the zymogrammes



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

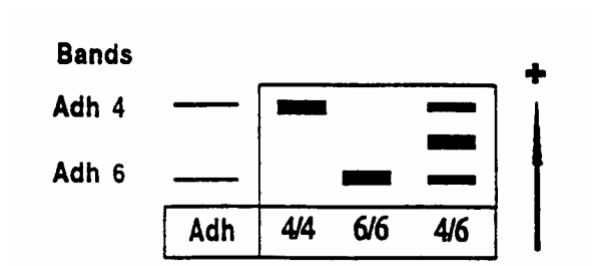
6.8 Recognition of the alleles encoding ADH

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



Description of the example inbred lines

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

[End of Annex and of document]