CPVO · OCVV
Community Plant Variety Office
Office Communautaire des Variétés Végétales

PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Zea mays L.

MAIZE

UPOV Species Code: ZEAAA_MAY

Adopted on 11/03/2010

Entry into force on 01/01/2010

I SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on general UPOV document TG/1/3 and UPOV Guideline TG/2/7 dated 1 April 2009 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to all varieties of ***Zea mays* L.**, excluding ornamental varieties.

II SUBMISSION OF SEED AND OTHER PLANT MATERIAL

1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of:

   - the closing date for the receipt of plant material;
   - the minimum amount and quality of plant material required;
   - the examination office to which material is to be sent.

   A sub-sample of the material submitted for test will be held in the reference collection as the definitive sample of the candidate variety.

   The applicant is responsible for ensuring compliance with any customs and plant health requirements.

2. Final dates for receipt of documentation and material by the Examination Office

   The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen (hereunder point 3).

   The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO if no plant material has been received. However if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Seed requirement

   The final dates for request for technical examination and sending of Technical Questionnaire as well as submission date of plant material by the applicant, and quantity of plant material to be supplied by the applicant are published on the CPVO website and in the S2 official gazette.

   Quality of seed: ..... The minimum requirements for germination capacity and analytical purity should not be less than the standards laid down for certified seed in Council Directive 66/402/EEC for maize and in Annex II of Council Directive 2002/55/EC for sweet corn and pop corn.

   Seed Treatment ..... The plant material must not have undergone any treatment unless the CPVO and the examination office allow or request such treatment. If it has been treated, full details of treatment must be given.

   Special requirements: ..... -

   Labelling of sample:..... - Species
   - File number of the application allocated by the CPVO
   - Breeder's reference
   - Examination reference (if known)
   - Name of applicant
   - The phrase "On request of CPVO"

III CONDUCT OF TESTS

1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation (EC) No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;
- varieties protected in other UPOV Member States;
- any other variety in common knowledge.
- in the case of hybrids, all components of hybrid varieties in common knowledge must be considered as part of the variety collection.

The composition of the variety collection in each Examination Office depends on the ecological conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

2. Material to be examined

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the reference collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate their work with other offices involved in DUS testing of maize. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

3. Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the Annex 1. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted. In the latter case, the CPVO should be informed. In addition, the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

In cases where the distinction of hybrid varieties is based on the distinction of parental lines, the description of the hybrid must at least include those characteristics of Annex 1 which are indicated by an H.

The list of characteristics derived from enzyme electrophoresis as in Annex 2 should only be used as a complement to other differences in morphological or physiological characteristics.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation (EC) No.874/2009, to insert additional characteristics and their expressions in respect of a variety.

4. Grouping of varieties

The varieties and candidates to be compared will be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups. The characteristics that could be used for grouping are the following (CPVO numbering; G for grouping in the table of characteristics):

a) Tassel: time of anthesis (characteristic 6)

b) Tassel: anthocyanin coloration at base of glume (characteristic 7)

c) Ear: anthocyanin coloration of silks (characteristic 14)

d) Plant: length (characteristic 22)

e) Ear: type of grain (characteristic 34)

f) Excluding varieties with ear type of grain: sweet: Ear: colour of dorsal side of grain (characteristic 37)

g) Ear: anthocyanin coloration of glumes of cob (characteristic 39)

5. Trial designs and growing conditions

The minimum duration of tests will normally be two independent growing cycles. Tests will be carried out under conditions ensuring normal growth. The size of the plots will be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing cycle.

The test design is a s follows:

As a minimum, each test should include a total of 40 plants in the case of inbred lines and single hybrids and 60 plants in the case of other hybrids and open-pollinated varieties, divided between two or more replicates.

Number of Plants / Parts of Plants to be Examined

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.

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.

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.

6. Special tests

In accordance with Article 83(3) of Council Regulation (EC) No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

7. Standards for decisions

a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of Article 7 of Council Regulation (EC) No. 2100/94.

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 Annex 1 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.

Qualitative characteristics:

In the case of characteristics which show discrete discontinuous states of expression, a difference between two varieties is clear if one or more of the respective characteristics have expressions which fall into two different states.

Quantitative characteristics:

Characteristics which show a continuous range of expression from one extreme to the other may be either measured or visually observed.

In the case of visually observed characteristics, a difference between two varieties is clear if the expression of the respective characteristics differs by at least the span of one note, taking into account the variability observed within the varieties.

If distinctness is assessed by the combined over years distinctness analysis (COYD) the difference between two varieties is clear if the respective characteristics are different at the 1% significance level or less ($p \leq 0.01$) in a test over either two or three years.

If distinctness is assessed using the t-test least significant difference the difference between two varieties is clear if it occurs with the same sign at the 1% significance level or less ($p \leq 0.01$) in two consecutive or two out of three growing cycles.

If the significance level or statistical methods proposed are not appropriate the method used should be clearly described.

b) **Uniformity**

For the assessment of uniformity of inbred lines and single-cross hybrids a population standard of 3% with an acceptance probability of 95% should be applied. In the case of a sample of 40 plants, the maximum number of off-types allowed would be 3. 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 in case of lack of uniformity on morphological level).

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.

In three-way cross hybrids and double-cross hybrids, characteristics may segregate with the effect that several states of expression occur side by side in a variety. Certain characteristics which from experience are known to give rise to such segregations in three-way cross hybrids and double-cross hybrids are identified with an "S" in the table of characteristics in Annex I.

If ear-rows are also used, a population standard of 3 % with an acceptance probability of 95 % should be applied. The candidate is considered to be sufficiently uniform if the number of off-type ear- rows does not exceed 1 in 6 ear-rows examined. For sown plots the above-mentioned population standard is applicable. By analogy this provision also applies to any plant progeny test.

With respect to the use of enzyme electrophoresis, the Office follows the actual UPOV approach as laid down in part I of Annex 2 hereto. If enzyme electrophoresis is used for testing distinctness, the same population standard and the same acceptance probability as for other characteristics should be applied. However, a sequential analysis approach could be applied to reduce the workload. Any plant in an inbred line should be considered as an out-cross where two or more loci are heterozygous with one allele of the locus of the inbred line (e.g. AX). All cases where one locus is heterozygous or where two foreign alleles are present at least one locus should be considered off-types.

c) **Stability**

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity.

Seed samples of further submissions included in any test must show the same expression of characteristics as the material originally supplied.

IV REPORTING OF RESULTS

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two growing cycles but in some cases three growing cycles may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive interim reports and final reports by the date agreed between the CPVO and the examination office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

V LIAISON WITH THE APPLICANT

If problems arise during the course of the test the CPVO should be informed so that the information can be passed on to the applicant. Subject to prior permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

The annual interim report as well as the final report shall be sent by the Examination Office to the CPVO.

VI ENTRY INTO FORCE

The present protocol enters into force on **01/01/2010.** Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the revised Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

In cases where the Office requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process to be carried out at the moment of this request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

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ANNEXES TO FOLLOW

ANNEX I PAGE

Legend:

(+) See explanations on the table of characteristics
(a)-(e) See explanations on the table of characteristics
H Characteristics to be included into the description of a hybrid as a minimum
G Grouping characteristic
(S) Possible segregation in three-way and double-cross hybrid varieties
PC Popcorn variety
SC Sweet corn variety
14-93 Decimal Code for the Growth Stages

Types of expression of characteristics:

QL Qualitative characteristic
QN Quantitative characteristic
PQ Pseudo-qualitative characteristic

Type of observation of characteristics:

MG Single measurement of a group of plants or parts of plants
MS Measurement of a number of individual plants or parts of plants
VG Visual assessment by a single observation of a group of plants or parts of plants
VS Visual assessment by observation of individual plants or parts of plants

When a method of observation is attributed to a certain characteristic, the first differentiation is made depending if the action taken is a visual observation (V) or a measurement (M).

The second differentiation deals with the number of observations the expert attributes to each variety, thus the attribution of either G or S.

If a single observation of a group consisting of an undefined number of individual plants is appropriate to assess the expression of a variety, we talk about a visual observation or a measurement made on a group of plants, thus we attribute the letter G (either VG or MG). If the expert makes more than one observation on that group of plants, the decisive part is that we have at the end only one data entry per variety which means that we have to deal with G (e.g. measurement of plant length on a plot – MG, visual observation of green colour of leaves on a plot – VG).

If it is necessary to observe a number of individual plants to assess the expression of a variety, we should attribute the letter S (thus either VS or MS). Single plant data entries are kept per variety for further calculations like the variety mean (e.g. measurement of length of ears – MS, visual observation of growth habit of single plants in grasses – VS). The number of individual plants to be observed in such cases is stated in section III.5.

ANNEX II

ANNEX III

ANNEX I

TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTIONS

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
1.	**1.**	**VG**	**First leaf: anthocyanin coloration of sheath**		
QN		**14**	absent or very weak	0674, Jubilee (SC)	1
		(S)	weak	MO17, Puma (SC)	3
			medium	F252, Gyöngymazsola (SC)	5
			strong	F244	7
			very strong		9
2.	**2.**	**VG**	**First leaf: shape of apex**		
(+)		**14**	pointed		1
PQ			pointed to rounded	0674	2
			rounded	Empire (SC), F816	3
			rounded to spatulate	F259, Merkur (SC)	4
			spatulate	EP1	5
3.	**3.**	**VG**	**Foliage: intensity of green colour**		
QN		**51-59**	light	W182E	1
			medium	W117, Empire (SC)	2
			dark	GSS 3287 (SC), W401	3
4.	**5.**	**VG**	**Leaf: angle between blade and stem**		
(+)		**65-69**	very small		1
QN		**(a)**	small	A188	3
H			medium	F66, GH2547 (SC)	5
			large	F186, Spirit (SC)	7
			very large		9

[1] The optimum stage of development for the assessment of each characteristic is indicated by a number in the second column. The stages of development denoted by each number are described in the Decimal Code for the growth stages (page 25).

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
5.	**6.**	**VG**	**Leaf: curvature of blade**		
(+)		**65-69**	absent or very slightly recurved	WD36	1
QN		**(a)**	slightly recurved	A654, Bonus (SC)	3
H			moderately recurved	W117, Jubilee (SC)	5
			strongly recurved	W79A	7
			very strongly recurved		9
6.	**8.**	**MG**	**Tassel: time of anthesis**		
(+)	*	**(b)**	very early		1
QN			very early to early	KW1069, Spirit (SC)	2
H			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
G			very late		9
7.	**9.**	**VG**	**Tassel: anthocyanin coloration at base of glume**		
(+)	*	**65-69**	absent or very weak	W117, Royalty (SC)	1
QN		**(S)**	weak	F66, Boston (SC)	3
H		**(b)**	medium	F107	5
			strong	EP1	7
G			very strong		9
8.	**10.**	**VG**	**Tassel: anthocyanin coloration of glumes excluding base**		
(+)		**65-69**	absent or very weak	F259, Empire (SC)	1
QN		**(S)**	weak	F2, Royalty (SC)	3
H		**(b)**	medium	WD36, Centurion (SC)	5
			strong	W79A	7
			very strong		9

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
9.	**11.**	**VG**	**Tassel: anthocyanin coloration of anthers**		
(+)		**(S)**	absent or very weak	A654, Empire (SC)	1
QN		**(b)**	weak	F2, Royalty (SC)	3
H			medium	W182E, Centurion (SC)	5
			strong		7
			very strong		9
10.	**12.**	**VG**	**Tassel: angle between main axis and lateral branches**		
(+)	*	**65-69**	very small		1
QN		**(c)**	small	F492	3
H			medium	EP1, Mv. Aranyos (SC)	5
			large	F186, Bonus (SC)	7
			very large		9
11.	**13.**	**VG**	**Tassel: curvature of lateral branches**		
(+)	*	**69**	absent or very slightly recurved	F257, El Toro (SC)	1
QN		**(S)**	slightly recurved	F816, Empire (SC)	3
H		**(c)**	moderately recurved	W182E, Bonus (SC)	5
			strongly recurved	F66	7
			very strongly recurved		9
12.	**14.**	**MS/VG**	**Tassel: number of primary lateral branches**		
QN	*	**65-75**	absent or very few	F7	1
H			few	F252, Mv. Aranyos (SC)	3
			medium	F244, Kokanee (SC)	5
			many	A188, Zenith (SC)	7
			very many	Suregold (SC)	9

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
13.	**15.**	**MG**	**Ear: time of silk emergence**		
(+)			very early	Mv. Aranyos (SC)	1
QN			very early to early	KW1069, Spirit (SC)	2
H			early	F257, Champ (SC)	3
			early to medium	F259, Royalty (SC)	4
			medium	F522, Bonus (SC)	5
			medium to late	A632	6
			late	B73	7
			late to very late	AM1513	8
			very late		9
14.	**16.**	**VG**	**Ear: anthocyanin coloration of silks**		1
QN	*	**65**	absent or very weak	F7, F195, Bonus (SC)	1
H		**(S)**	weak	F257, El Toro (SC)	3
			medium	F244, Gyöngymazsola (SC)	5
			strong	W401	7
G			very strong		9
15.	**17.**	**VG**	**Stem: anthocyanin coloration of brace roots**		
(+)		**61 - 79**	absent or very weak	F16, Jubilee (SC)	1
QN		**(S)**	weak	W117, Puma (SC)	3
H			medium	WD36, El Toro (SC)	5
			strong	EP1	7
			very strong		9
16.	**18.**	**VG**	**Tassel: density of spikelets**		
QN		**61-71**	moderately lax	F16	3
		(b)	medium	EP1, Royalty (SC)	5
			moderately dense	F259, Empire (SC)	7

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
17.	**19.**	**VG**	**Leaf: anthocyanin coloration of sheath**		
(+)		**71-75**	absent or very weak	W401, Jubilee (SC)	1
QN		**(S)**	weak	F107	3
			medium	F257	5
			strong	EP1	7
			very strong		9
18.	**20.**	**VG**	**Stem: anthocyanin coloration of internodes**		
(+)		**71-75**	absent or very weak	F259, Jubilee (SC)	1
QN		**(S)**	weak	F816	3
			medium	W79A	5
			strong	F257	7
			very strong		9
19.	**21.**	**MS**	**Tassel: length of main axis above lowest lateral branch**		
(+)		**71-75**	very short		1
QN			short	EP1	3
			medium	F244, Bonus (SC°	5
			long	F492, Empire (SC)	7
			very long		9
20.	**22.**	**MS**	**Tassel: length of main axis above highest lateral branch**		
(+)	*****	**71-75**	very short		1
QN			short	EP1	3
H			medium	W182E	5
			long	F492	7
			very long		9

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
21.	**23.**	**MS**	**Tassel: length of lateral branch**		
QN		**71-75**	very short		1
		(c)	short	EP1	3
			medium	A632	5
			long	F492	7
			very long		9
22.1.	**24.1**	**MS**	**Only inbred lines and varieties with ear type of grain: sweet or pop: Plant: length**		
(+)	*	**75-85**	very short	F7	1
QN			short	W117, Spirit (SC)	3
			medium	F244, Puma (SC)	5
			long	WD36, Royalty (SC)	7
G			very long	Enterprise (SC)	9
22.2	**24.2**	**MS**	**Only hybrids and open-pollinated varieties, excluding varieties with ear type of grain: sweet or pop: Plant: length**		
(+)	*	**75-85**	very short		1
QN			short	PR39D23	3
H			medium	PR37Y12	5
			long	DKC5166	7
G			very long		9
23.	**25.**	**MG**	**Plant: ratio height of insertion of peduncle of upper ear to plant length**		
(+)		**75-85**	very small	Gyöngymazsola (SC)	1
QN			small	F816, Spirit (SC)	3
H			medium	F252, Royalty (SC)	5
			large	F481	7
			very large		9

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
24.	**26**	**MS**	**Leaf: width of blade**		
QN		**75-85**	very narrow		1
		(a)	narrow	F16, Champ (SC)	3
			medium	F244, Empire (SC)	5
			wide	F481, Centurion (SC)	7
			very wide		9
25.	**27.**	**VG**	**Peduncle: length**		
QN		**75-85**	very short		1
			short	F259, Centurion (SC)	3
			medium	A654, Jubilee (SC)	5
			long	F107	7
			very long		9
26.	**28.**	**MS**	**Ear: length**		
(+)	*	**92-93**	very short		1
QN		**sweet corn:**	short	F2	3
H		**75-79**	medium	A654, Spirit (SC)	5
			long	MO17, Empire (SC)	7
			very long		9
27.	**29.**	**MS**	**Ear: diameter (in middle)**		
QN		**92-93**	very small		1
H		**sweet corn:**	small	F7	3
		75-79	medium	W117	5
			large	F481, Centurion (SC)	7
			very large	Empire (SC)	9

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
28.	**30.**	**VG**	**Ear: shape**		
(+)		**92-93**	conical	F16, Wombat (SC)	1
QN		**sweet corn:**	conico-cylindrical	F816, Centurion (SC)	2
		75-79	cylindrical	F66, GH2547 (SC)	3
29.	**31.**	**MS**	**Ear: number of rows of grain**		
QN	**QN**	**92-93**	very few		1
H		**sweet corn:**	few	F257	3
		75-93	medium	F16, Dessert 73 (SC)	5
			many	B73, Bonus (SC)	7
			very many		9
30. (+)	**32.**	**VG**	**Only varieties with ear type of grain: sweet or waxy: Ear: number of colours of grains**		
QL		**75-79 (S)**	one	Jubilee (SC)	1
H		**(e)**	two	Eolrukchal-ilho, Serendipity (SC)	2
31.	**33.**	**VG**	**Only varieties with ear type of grain: sweet: Grain: intensity of yellow colour**		
QN	*****	**75-79**	light	Gyöngymazsola (SC)	3
H		**(e)**	medium	Royalty (SC)	5
			dark	Kokanee (SC)	7
32.	**34.**	**VG**	**Only varieties with ear type of grain: sweet: Grain: length**		
QN		**75-79**	short		3
H		**(d)**	medium	Boston (SC)	5
			long	GH5704 (SC)	7
33.	**35.**	**VG**	**Only varieties with ear type of grain: sweet: Grain: width**		
(+)		**75-79**	narrow	Bonus (SC)	3
QN		**(d)**	medium	Jubilee (SC)	5
H			broad	Mv. Aranyos (SC)	7

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
34.	**36.**	**VG**	**Ear: type of grain**		
(+)	*	**92-93 (S)**	flint	F2	1
QL		**(d)**	flint-like	F252	2
H		**(e)**	intermediate	F107	3
			dent-like	A654	4
			dent	W182E	5
			sweet	Jubilee (SC)	6
			pop	Iowa Pop (SC)	7
			waxy		8
G			flour		9
35.	**37.**	**VG**	**Only varieties with ear type of grain: sweet: Ear: shrinkage of top of grain**		
(+)	*	**92-93**	weak	Zarja (SC)	1
QN		**(d)**	medium	Merkur (SC)	3
H		**(e)**	strong	Dessert 73 (SC)	5
36.	**38.**	**VG**	**Ear: colour of top of grain**		
PQ	*	**92-93**	white	A188, Purple white (SC), Snowbelle (SC)	1
H		**(S)**	yellowish white		2
		(d)	yellow	F259	3
		(e)	yellow orange	F2, Gyöngymazsola (SC)	4
			orange	F257, GH2547 (SC)	5
			red orange	Dynasty (SC)	6
			red		7
			purple		8
			brownish	Zenith (SC)	9
			blue black	Miheukchal	10

CPVO N°	UPOV N°	Stage[1]	Characteristics	Examples	Note
37.	**39.**	**VG**	**<u>Excluding varieties with ear type of grain: sweet</u>: Ear: colour of dorsal side of grain**		
PQ	*	**92-93**	white	F481	1
H		**(S)**	yellowish white	A188	2
		(d)	yellow		3
		(e)	yellow orange	F66	4
			orange	EP1	5
			red orange		6
			red		7
			purple		8
			brownish		9
G			blue black		10
38.	**40.**	**VG**	**<u>Only varieties with ear type of grain: pop:</u> Type of popped grain**		
(+)		**93**	butterfly	Robust 97461 (PC)	1
QN			intermediate		2
H			globular	Robust 90252 (PC)	3
39.	**41.**	**VG**	**Ear: anthocyanin coloration of glumes of cob**		
(+)	*	**93**	absent or very weak	F2, F257	1
QN		**(S)**	weak	F252	3
H			medium	W117	5
			strong	A632	7
G			very strong		9

EXPLANATIONS AND METHODS

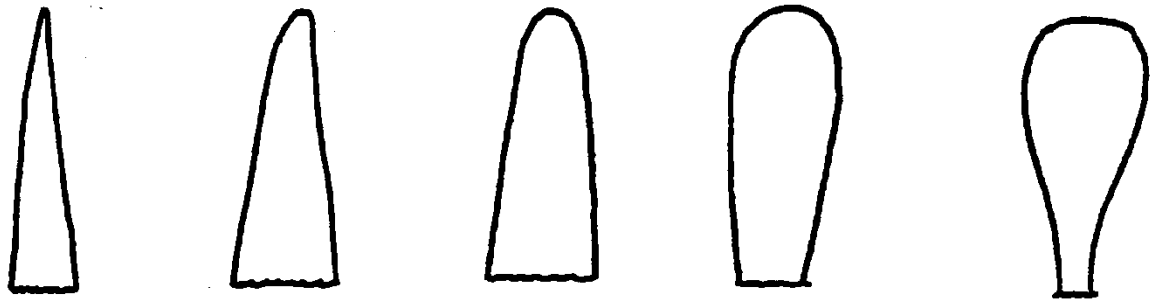
1 Explanations covering several characteristics

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 sweet corn and popcorn varieties, cross-pollination should be avoided.

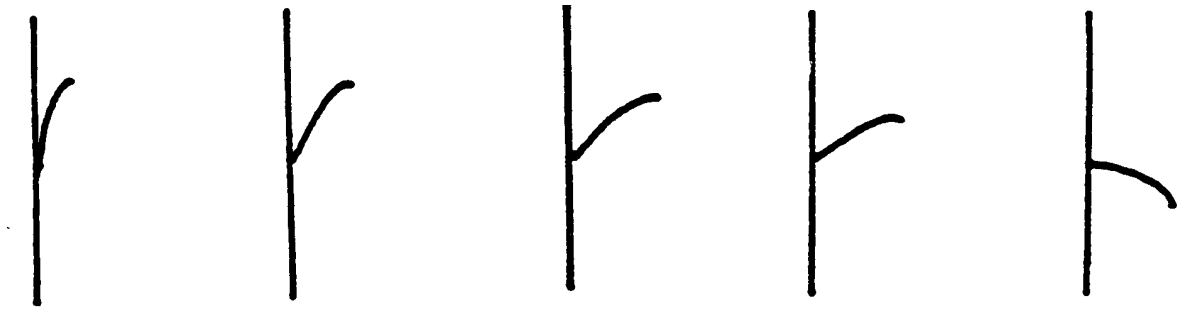
2 Explanations for individual characteristics

Ad. 2: First leaf: shape of apex



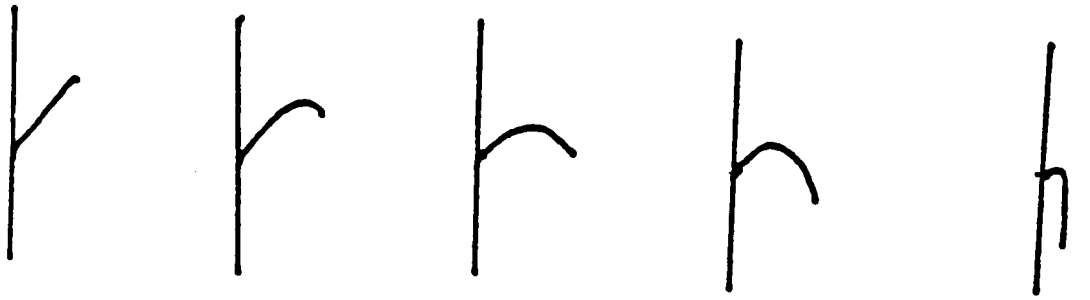
1	2	3	4	5
pointed	pointed to rounded	rounded	round to spatulate	spatulate

Ad. 4: Leaf: angle between blade and stem
Ad. 10: 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. 5: Leaf: curvature of blade
Ad. 11: Tassel: curvature of lateral branches

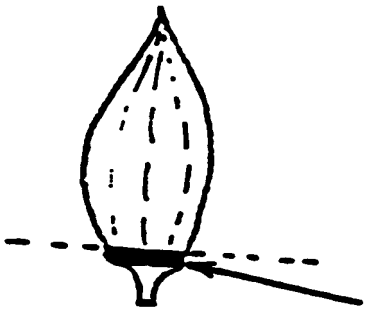


1	3	5	7	9
absent or very slightly recurved	slightly recurved	moderately recurved	strongly recurved	very strongly recurved

Ad. 6: 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. 7: Tassel: anthocyanin coloration at base of glume
Ad. 8: Tassel: anthocyanin coloration of glumes excluding base



base of glume

Ad. 9: Tassel: anthocyanin coloration of anthers

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

Ad. 13: Ear: time of silk emergence

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

Ad. 15: Stem: anthocyanin coloration of brace roots

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

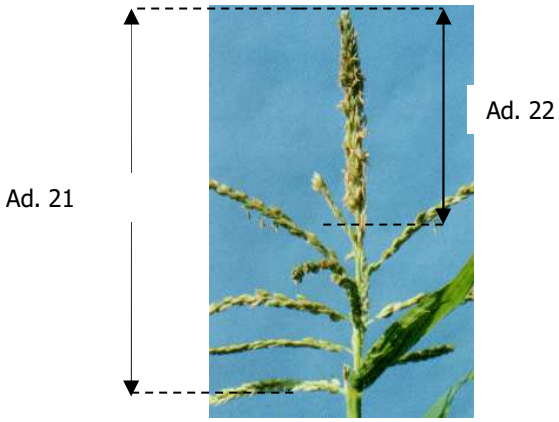
Ad. 17: Leaf: anthocyanin coloration of sheath

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

Ad. 18: Stem: anthocyanin coloration of internodes

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

Ad. 19: Tassel: length of main axis above lowest lateral branch
Ad. 20: Tassel: length of main axis above highest lateral branch

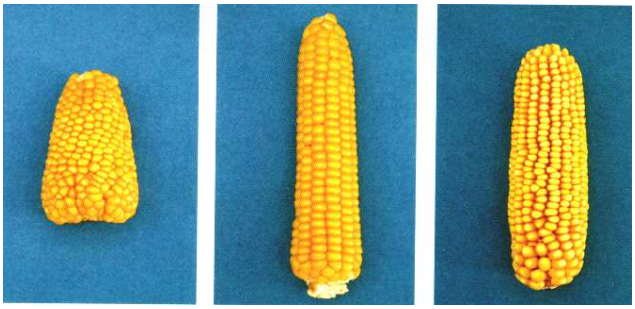


Ad. 22.1: Only inbred lines and varieties with ear type of grain: sweet or pop: Plant: length
Ad. 22.2: Only hybrids and open-pollinated varieties, excluding varieties with ear type of grain: sweet or pop: Plant: length
Ad. 23: Plant: ratio height of insertion of peduncle of upper ear to plant length

The plant length should be observed including the tassel.

Ad. 26: Ear: length

Ad. 28: Ear: shape



1 conical | 2 conico-cylindrical | 3 cylindrical

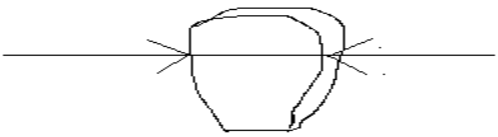
Ad. 30: Only varieties with ear type of grain: sweet or waxy: Ear: number of colours of grains

1
one

2
two

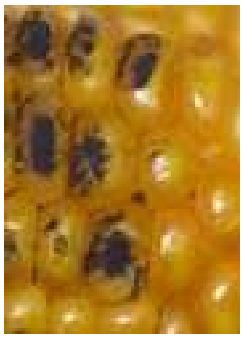
Bicolour varieties have yellow and white grains on their ears. Cross pollination should be avoided particularly between white, bicolour and yellow grain coloured varieties.

Ad. 33: Only varieties with ear type of grain: sweet: Grain: width



Ad. 34: 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 (blue black coloration of other types of grain). Iodine staining test   waxy non waxy
9	flour	completely soft endosperm, grain round or slightly indented on crown

Ad. 35: Only varieties with ear type of grain: sweet: Ear: shrinkage of top of grain


1
weak


3
medium


5
strong

In order to obtain uniform expressions of the grains on the ear, cross pollination between varieties should be avoided, e.g. by a special trial design. Cross pollination can cause irregular expressions of the grains and may lead to a wrong interpretation that the variety is not uniform.

Ad. 38: Only varieties with ear type of grain: pop: Type of popped grain

Ear 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. 39: 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.

*3 Decimal Code for the Growth Stages**

CODE	GENERAL DESCRIPTION
	Seedling growth
14	4 leaves unfolded
	Tillering
	Stem elongation
	Booting
	Inflorescence emergence
51 (♂,♀)	Inflorescence just visible
59	Emergence of inflorescence completed
(♂,♀)	
	Anthesis
61	Beginning of anthesis
65	Anthesis halfway
69	Anthesis complete
	Milk development
71	Caryopsis watery ripe

CODE	GENERAL DESCRIPTION
73	Early milk
75	Medium milk
79 (1)	Grains have reached final size
	Dough development
85	Soft dough
	Ripening
92	Caryopsis hard (can no longer be dented y thumbnail)
93	Caryopsis loosening in daytime

* 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.

ANNEX II

PROTEIN ELECTROPHORESIS

Additional Useful Explanations

TABLE OF CONTENTS

Part I

Introduction

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

For the analysis of 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
40.	**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	
41.	**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
42.	**Allele expression at locus Mdh 3**	Genotype 16/16	F252	1
QL		Genotype 18/18	CO 158	2
		Genotype 16/18	Figaro	3
43.	**Allele expression at locus Mmm**	Genotype M/M	F252	1
		Genotype M/m		
QL		Genotype m/m	86 N 42	2
44.	**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	

	Characteristics		Examples	Note
45.	**Allele expression at loci Idh1 + Idh 2**	Genotype 4/4 + 4/4 Genotype 4/6 + 4/4	A239	1
QL		Genotype 4/4 + 6/6	CM7	2
		Genotype 6/6 + 4/4	F1110	3
		Genotype 6/6 + 6/6 Genotype 4/6 + 6/6	CO 158 Bonny	4
		Genotype 4/4 + 4/6 Genotype 4/6 + 4/6	Axon Loft	5
		Genotyp 6/6 + 4/6		6
46.	**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
47.	**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

	Characteristics		Examples	Note
48.	**Allele expression at locus Pgi 1**	Genotype 4/4	A239	1
QL		Genotype 5/5	A632	2
		Genotype 4/5	Artist	3
49.	**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
50.	**Allele expression at locus Dia 1**	Genotype 8/8	F 2	1
QL		Genotype 12/12	CO 158	2
		Genotype 8/12	Bastion	3
51.	**Allele expression at locus Dia2**	Genotype 4/4	F 2	1
QL		Genotype 6/6	34 M 838	2
		Genotype 4/6	31 N 6	3
52.	**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 litres 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

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)

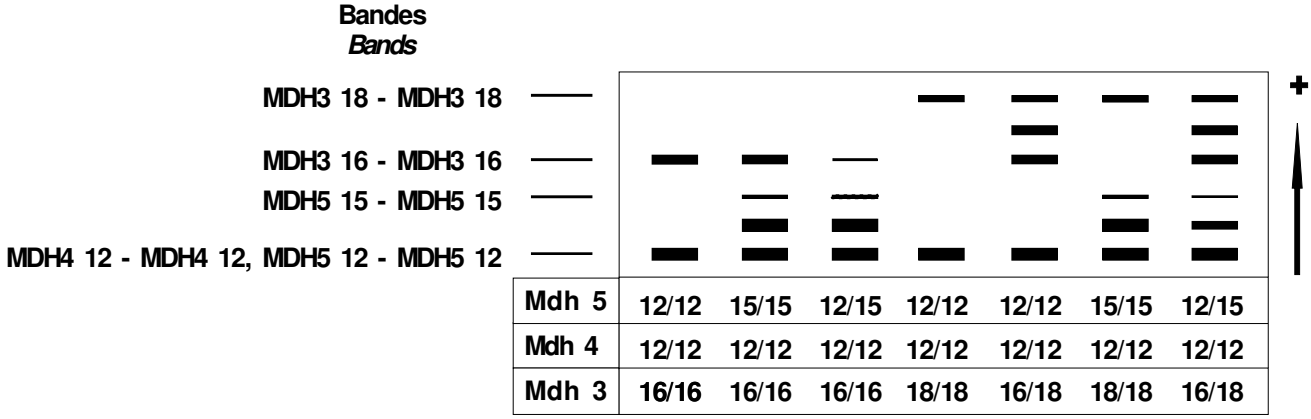
- There are interactions between the products of the genes (polypeptide subunits) on the one hand, encoded by Mdh1, Mdh2, Mdh3, and on the other hand, encoded by Mdh4 and Mdh5.

	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.

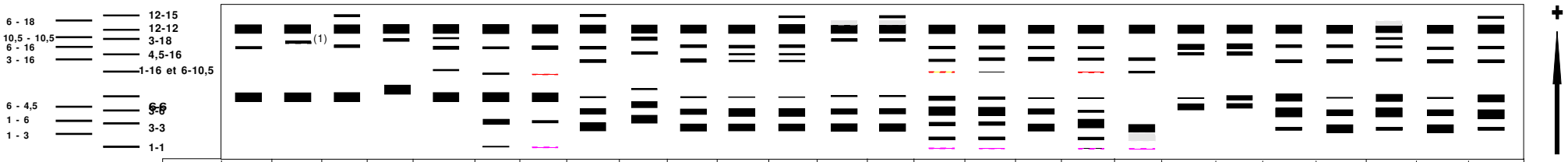
Zymograms of MDH from maize coleoptile in pH 5.0 buffer system:



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:

Bandes/Bands



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

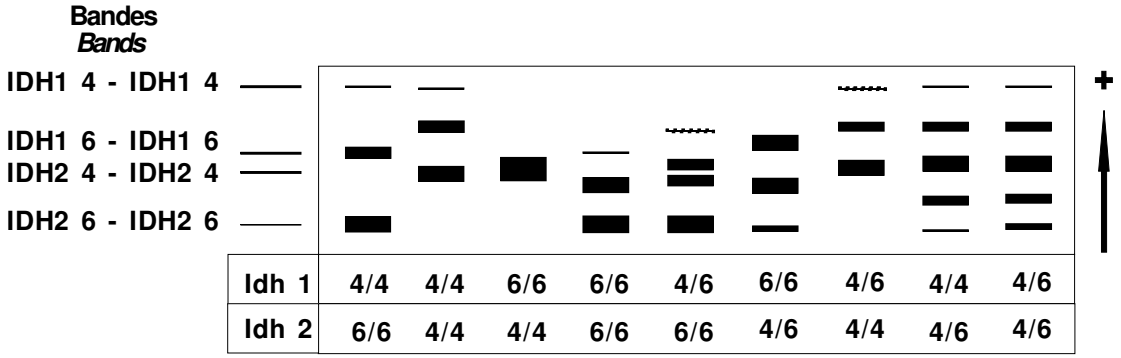
6.2.1 Genetic interpretation of the zymogrammes

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

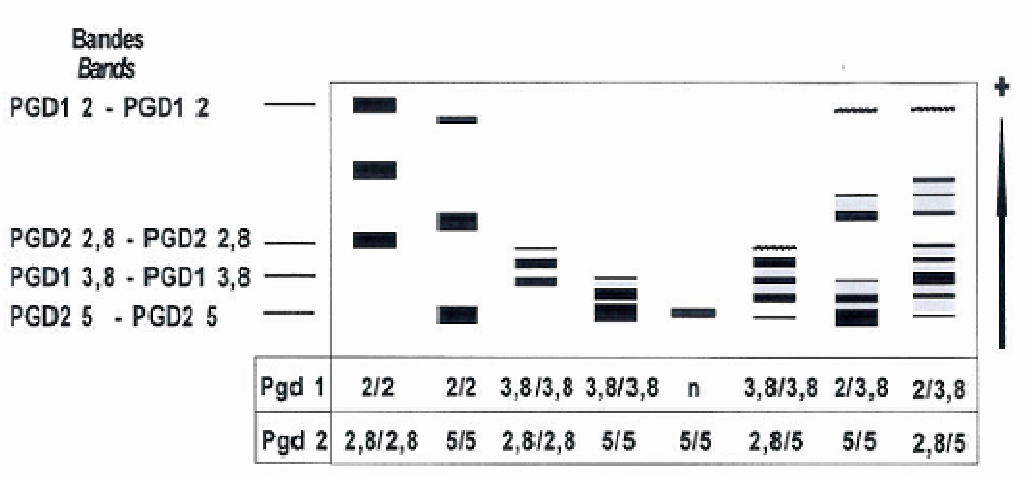
6.3.1 Genetic interpretation of the zymogrammes

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.

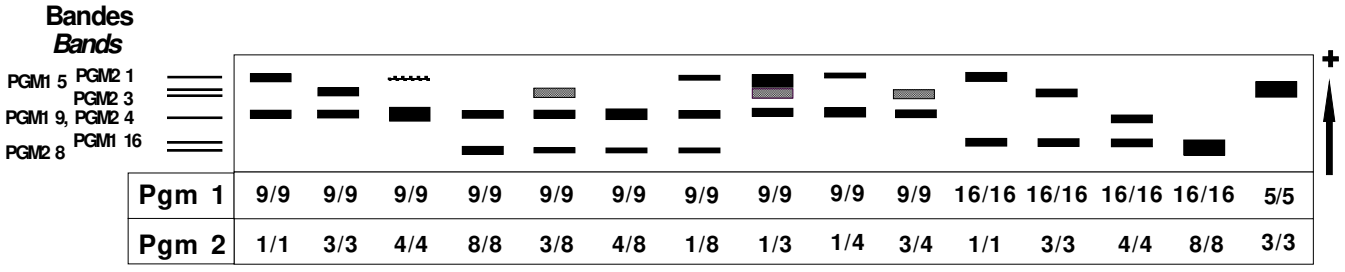
6.4 Recognition of the alleles encoding PGM

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.

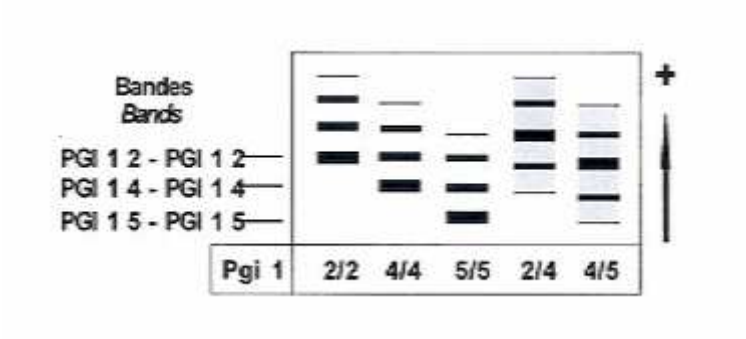
6.5 Recognition of the alleles encoding PGI

6.5.1 Genetic interpretation of the zymogrammes

	Quaternary	**Chromosomal**		
Enzyme	**structure**	**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



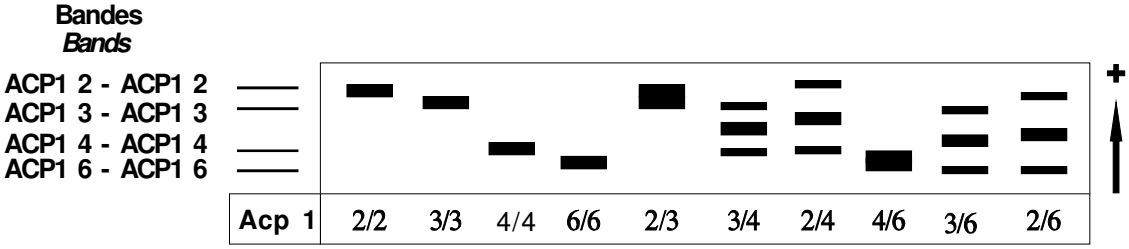
6.6 Recognition of the alleles encoding ACP

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.

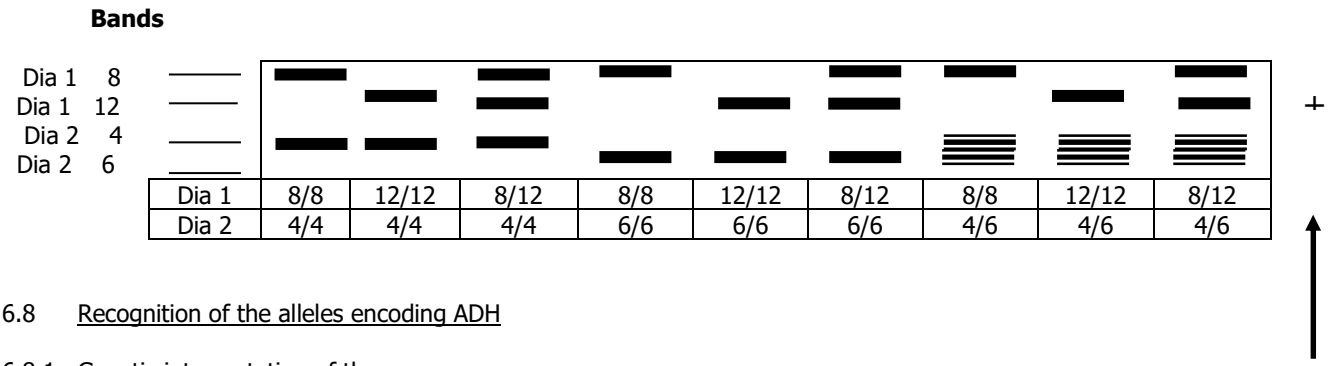
6.7 Recognition of the alleles encoding DIA

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

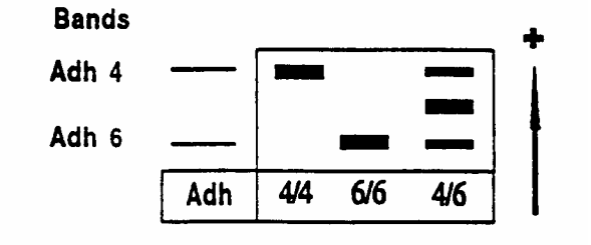
6.8 Recognition of the alleles encoding ADH

6.8.1 Genetic interpretation of the zymogrammes

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

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

6.8.2 Schematization of the zymogrammes



Description of the example inbred lines

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

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ANNEX III

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/002/3