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TC/33/6

English only

DATE: August 18, 1996

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL COMMITTEE**Thirty-Third Session
Geneva, October 16 to 18, 1996****AMENDMENTS OF THE TEST GUIDELINES FOR WHEAT (TG/3/11), MAIZE (TG/2/6)
AND BARLEY (TG/19/10)***Document prepared by the Office of the Union*

1. During its last session, the Technical Working Party for Agricultural Crops (TWA) amended the above Test Guidelines as follows:

Test Guidelines for Wheat (TG/3/11)

(a) Addition of a further state of expression referring to bands 6.1 + 22 in characteristic 28 on page 3 of the Annex with the corresponding changes in the explanations (pages 7 and 8 of the Annex in English).

(b) Correction of an error in the Notes of characteristic 21 in the Table of Characteristics on page 15. The Notes should, as correctly stated in the explanations, read: 1, 3, 5, 7, 9 instead of 1, 2, 3, 4, 5.

Test Guidelines for Maize (TG/2/6)

(a) Correction of Locus PGM1 and PGM2 in characteristic 42.1 and 42.2 on pages 4 and 5 of the Annex in English with the corresponding changes in the explanations under 6.4.2 (page 14 of the Annex in English).

(b) Correction of some further linguistic errors and missing paragraphs in some languages (according to the proposal of the Editorial Committee (English: IV(6), French and German: III(6)).

Test Guidelines for Barley (TG/19/10)

Addition of two new C-hordein alleles in characteristic 31 and four B-hordein alleles in characteristic 32 on pages 3 and 4 of the Annex in English with the corresponding changes in the explanations (pages 8, 10, 11 and 12 of the Annex in English).

2. The amended pages are reproduced as the following pages of this document:

Wheat: pages 3 - 6
Maize: pages 7 - 10
Barley: pages 11 - 15.

3. *The Technical Committee is invited to approve the above changes and the printing of a corrected version of the above Test Guidelines.*

EXTRACT FROM TEST GUIDELINES FOR WHEAT (TG/3/11)

Page 15

Characteristics Caractères Merkmale	Stage ¹⁾ Stade ¹⁾ Stadium ¹⁾	English	français	deutsch	Example Varieties Exemples Beispielssorten	Note
17. Apical rachis segment: (+) hairiness of convex surface	80-92	absent or very weak	nulle ou très faible	fehlend oder sehr gering	Soissons; -	1
	VS	weak	faible	gering	Slejpner; Furio	3
	Article terminal du rachis: pilosité de la face externe	medium	moyenne	mittel	Beaver; Rock	5
	Oberstes Spindelglied: äussere Behaarung	strong	forte	stark	Apollo; Axona	7
		very strong	très forte	sehr stark	Carat; -	9
18. Lower glume: shoulder (+) width (spikelet in mid-third of ear)	80-92	absent or very narrow	nulle ou très étroite	fehlend oder sehr schmal	Courtot; -	1
	VS	narrow	étroite	schmal	Soissons; Wim	3
	Glume inférieure: largeur de la troncature (épillé du tiers moyen de l'épi)	medium	moyenne	mittel	Sideral; Furio	5
	Hüllspelze: Schulter- breite (Ährchen im middle- ren Drittel der Ähre)	broad	large	breit	Castan; Filou	7
		very broad	très large	sehr breit	Abo	9
19. Lower glume: shoulder (+) shape (as for 18)	80-92	sloping	inclinée	abfallend	Courtot; -	1
	VS	slightly sloping	légèrement inclinée	leicht abfallend	Forby; Ventura	3
	Glume inférieure: forme de la troncature (comme pour 18)	straight	droite	gerade	Herzog; Prinqual	5
	Hüllspelze: Schulterform (wie unter 18)	elevated	échancrée	gehoben	Beaver; Adonis	7
		strongly elevated with 2nd point present	fortement échancrée avec présence d'un 2ème bec	stark gehoben mit vor- handener zweiter Spitze	Farnese; -	9
20. Lower glume: beak length (as for 18)	80-92	very short	très court	sehr kurz	Aladin; Sunnan	1
	VS	short	court	kurz	Sideral; Axona	3
	Glume inférieure: longueur du bec (comme pour 18)	medium	moyen	mittel	Recital; Furio	5
	Hüllspelze: Zahnlänge (wie unter 18)	long	long	lang	Soissons; Tejo	7
		very long	très long	sehr lang	Courtot; Prinqual	9
21. Lower glume: beak (+) shape (as for 18)	80-92	straight	droit	gerade	Festival; Lobo	1
	VS	slightly curved	légèrement coudé	leicht gebogen	Slejpner; Furio	3
	Glume inférieure: forme du bec (comme pour 18)	moderately curved	demi- coudé	mittel gebogen	Courtot; Rock	5
	Hüllspelze: Zahnform (wie unter 18)	strongly curved	fortement coudé	stark gebogen	Arum; -	7
		geniculate	genouillé	geknickt	-; -	9

Annex, Part II (Page 3 of the English Version)

Characteristics Caractères Merkmale	Stage ¹⁾ Stade ¹⁾ Stadium ¹⁾	English	français	deutsch	Example Varieties Exemples Beispielssorten	Note
27. (+) Glutenin composition: allele expression at locus Glu-A1		band 1	bande 1	Bande 1	Kadett	1
		band 2*	bande 2*	Bande 2*	Courtot	2
	Gluténine: expression de l'allèle occupant le locus Glu-A1	no band	pas de bande	Nullbande	Talent	3
Glutenin-Zusammensetzung: Allel-Ausprägung im Locus Glu-A1						
28. (+) Glutenin composition: allele expression at locus Glu-B1		bands 6 + 8	bandes 6 + 8	Banden 6 + 8	Norman	1
		bands 7 + 8	bandes 7 + 8	Banden 7 + 8	Courtot	2
	Composition de la gluténine: expression de l'allèle occupant le locus Glu-B1	bands 7 + 9	bandes 7 + 9	Banden 7 + 9	Kadett	3
		band 7 (or 7 + 9 in the presence of bands 5 + 10 of char. 29)	bande 7 (ou 7 + 9 en présence des bandes 5 + 10 du car. 29)	Bande 7 (oder 7 + 9 in Gegenwart der Banden 5 + 10 des Merkm. 29)	Okapi	4
	Glutenin-Zusammensetzung: Allel-Ausprägung im Locus Glu-B1	bands 13 + 16	bandes 13 + 16	Banden 13+ 16	Carala	5
		bands 14 + 15	bandes 14 + 15	Banden 14 + 15	Troll	6
		bands 17 + 18	bandes 17 + 18	Banden 17 + 18	Moulin	7
		band 20	bande 20	Bande 20	Figaro	8
		bands 6.1 + 22	bandes 6.1 + 22	Banden 6.1 + 22	Schwabenkorn	9
29. (+) Glutenin composition: allele expression at locus Glu-D1		bands 2 + 12	bandes 2 + 12	Banden 2 + 12	Courtot	1
		bands 3 + 12	bandes 3 + 12	Banden 3 + 12	Norman	2
	Composition de la gluténine: expression de l'allèle occupant le locus Glu-D1	bands 4 + 12	bandes 4 + 12	Banden 4 + 12	Talent	3
		bands 5 + 10	bandes 5 + 10	Banden 5 + 10	Kadett	4
Glutenin-Zusammensetzung: Allel-Ausprägung im Locus Glu-D1						

Characteristic 29: Glu-D1 locus

	Example variety (Courtot)	Note			
		1 (a)	2 (b)	3 (c)	4 (d)
1 (113)---					
2/2* (108)---	2/2*---	2---			
3 (107)---			3---		
4 (106)---				4---	
5 (105)---					5---
6 (100)---					
6.1 (99)---					
7 (98)---	7 ---				
13/14/ 20 (94)---					
15 (91)---					
16/ (90)---					
17/18 89.5)					
22 (87)---					
8 (86)---	8 ---				
9/10 (83)---					10---
12 (80)---	12 ---	12---	12---	12---	

Note: Certain bands (e.g. bands 9 and 10) have similar molecular weights. This leads to the fact that in the presence of bands 5 + 10 of characteristic 29 two states of expression of characteristic 28, band 7 and bands 7 + 9, cannot be differentiated from one another. Therefore, in the presence of bands 5 + 10 of characteristic 29, Note 4 of characteristic 28 could be either band 7 or bands 7 + 9. Other bands having similar molecular weights can be differentiated from one another by their known association with other bands. For characteristic 28, band 13 is always associated with band 16 and band 14 with band 15 while band 40 remains alone.

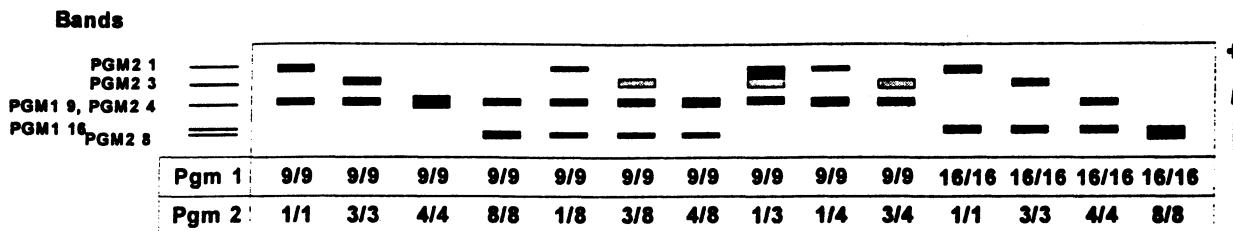
EXTRACT FROM TEST GUIDELINES FOR MAIZE (TG/2/6)

Annex, Pages 4 and 5 of the English Version

Characteristics Caractères Merkmale	English	français	deutsch	Example Varieties Exemples Beispielssorten	Note	
40. Allele expression at loci Idh 1 + Idh 2 Expression de l'allèle occupant les loci Idh 1 + Idh 2 Allel-Ausprägung in Loci Idh 1 + Idh 2	Genotype 4/4 + 4/4	Génotype 4/4 + 4/4	Genotyp 4/4 + 4/4	A 239	1	
	Genotype 4/6 + 4/4	Génotype 4/6 + 4/4	Genotyp 4/6 + 4/4			
	Genotype 4/4 + 6/6	Génotype 4/4 + 6/6	Genotyp 4/4 + 6/6	CM 7	2	
	Genotype 6/6 + 4/4	Génotype 6/6 + 4/4	Genotyp 6/6 + 4/4	F 1110	3	
	Genotype 6/6 + 6/6	Génotype 6/6 + 6/6	Genotyp 6/6 + 6/6	Co 158	4	
	Genotype 4/6 + 6/6	Génotype 4/6 + 6/6	Genotyp 4/6 + 6/6	Bonny		
	Genotype 4/4 + 4/6	Génotype 4/4 + 4/6	Genotyp 4/4 + 4/6	Axon	5	
	Genotype 4/6 + 4/6	Génotype 4/6 + 4/6	Genotyp 4/6 + 4/6	Loft		
	41. Allele expression at loci Pgd 1 + Pgd 2 Expression de l'allèle occupant les loci Pgd 1 + Pgd 2 Allel-Ausprägung in Loci Pgd 1 + Pgd 2	Genotype 2/2 + 5/5	Génotype 2/2 + 5/5	Genotyp 2/2 + 5/5	W 401	1
		Genotype 2/2 + 2.8/2.8	Génotype 2/2 + 2.8/2.8	Genotyp 2/2 + 2.8/2.8		2
Genotype 3.8/ 3.8 + 2.8/2.8		Génotype 3.8/ 3.8 + 2.8/2.8	Genotyp 3.8/ 3.8 + 2.8/2.8	A 632	3	
Genotype 3.8/3.8 + 5/5		Génotype 3.8/3.8 + 5/5	Genotyp 3.8/3.8 + 5/5	F 252	4	
Genotype 3.8/ 3.8 + 2.8/5		Génotype 3.8/ 3.8 + 2.8/5	Genotyp 3.8/ 3.8 + 2.8/5	Tekila		
Genotype n/n + 5/5		Génotype n/n + 5/5	Genotyp n/n + 5/5	H 108	5	
Genotype 2/3.8 + 5/5		Génotype 2/3.8 + 5/5	Genotyp 2/3.8 + 5/5	Bekefix	6	
Genotype 2/3.8 + 2.8/5		Génotype 2/3.8 + 2.8/5	Genotyp 2/3.8 + 2.8/5	Furio		
42.1 <u>Inbred lines only:</u> allele expression at loci Pgm 1 + Pgm 2 <u>Seulement lignées:</u> expression de l'allèle occupant les loci Pgm 1 + Pgm 2 <u>Nur Inzuchtlinien:</u> Allel-Ausprägung in Loci Pgm 1 + Pgm 2		Genotype 9/9 + 1/1	Génotype 9/9 + 1/1	Genotyp 9/9 + 1/1	F 2	1
		Genotype 9/9 + 3/3	Génotype 9/9 + 3/3	Genotyp 9/9 + 3/3	F 16	2
	Genotype 9/9 + 4/4	Génotype 9/9 + 4/4	Genotyp 9/9 + 4/4	A 632	3	
	Genotype 9/9 + 8/8	Génotype 9/9 + 8/8	Genotyp 9/9 + 8/8	Mo 17	4	
	Genotype 16/16 + 4/4	Génotype 16/16 + 4/4	Genotyp 16/16 + 4/4			
	Genotype 16/16 + 1/1	Génotype 16/16 + 1/1	Genotyp 16/16 + 1/1		5	
	Genotype 16/16 + 3/3	Génotype 16/16 + 3/3	Genotyp 16/16 + 3/3			
	Genotype 16/16 + 8/8	Génotype 16/16 + 8/8	Genotyp 16/16 + 8/8		6	

Characteristics Caractères Merkmale	English	français	deutsch	Example Varieties Exemples Beispielssorten	Note
42.2 <u>Hybrids and open-pollinated varieties only</u> : allele expression at loci Pgm 1 + Pgm 2 <u>Seulement hybrides et variétés à fécondation libre</u> : expression de l'allèle occupant les loci Pgm 1 + Pgm 2 <u>Nur Hybriden und frei-abblühende Sorten</u> : Allel-Ausprägung in Loci Pgm 1 + Pgm 2	Genotype 9/9 + 1/1	Génotype 9/9 + 1/1	Genotyp 9/9 + 1/1	Robin	1
	Genotype 9/9 + 1/3	Génotype 9/9 + 1/3	Genotyp 9/9 + 1/3		
	Genotype 9/9 + 3/3	Génotype 9/9 + 3/3	Genotyp 9/9 + 3/3	Figaro	
	Genotype 9/9 + 3/4	Génotype 9/9 + 3/4	Genotyp 9/9 + 3/4		
	Genotype 9/9 + 4/4	Génotype 9/9 + 4/4	Genotyp 9/9 + 4/4	Axon	
	Genotype 9/9 + 1/4	Génotype 9/9 + 1/4	Genotyp 9/9 + 1/4		
	Genotype 9/9 + 8/8	Génotype 9/9 + 8/8	Genotyp 9/9 + 8/8	Occitan	2
	Genotype 9/9 + 3/8	Génotype 9/9 + 3/8	Genotyp 9/9 + 3/8		
	Genotype 9/9 + 4/8	Génotype 9/9 + 4/8	Genotyp 9/9 + 4/8		
	Genotype 9/9 + 1/8	Génotype 9/9 + 1/8	Genotyp 9/9 + 1/8		
Genotype 16/16 + 1/1	Génotype 16/16 + 1/1	Genotyp 16/16 + 1/1		4	
Genotype 16/16 + 1/3	Génotype 16/16 + 1/3	Genotyp 16/16 + 1/3			
Genotype 16/16+ 3/3	Génotype 16/16 + 3/3	Genotyp 16/16 + 3/3			
Genotype 16/16 + 8/8	Génotype 16/16+ 8/8	Genotyp 16/16 + 8/8		5	
43. Allele expression at locus Pgi 1 Expression de l'allèle occupant le locus Pgi 1 Allel-Ausprägung in Locus Pgi 1	Genotype 4/4	Génotype 4/4	Genotyp 4/4	A 239	1
	Genotype 5/5	Génotype 5/5	Genotyp 5/5	A 632	2
	Genotype 4/5	Génotype 4/5	Genotyp 4/5	Artist	3
44.1 <u>Inbred lines only</u> : Allele expression at locus Acp 1 <u>Seulement lignées</u> : expression de l'allèle occupant le locus Acp 1 <u>Nur Inzuchtlinien</u> : Allel-Ausprägung in Locus Acp 1	Genotype 2/2	Génotype 2/2	Genotyp 2/2	F 2	1
	Genotype 3/3	Génotype 3/3	Genotyp 3/3	A 239	2
	Genotype 4/4	Génotype 4/4	Genotyp 4/4	A 632	3
	Genotype 6/6	Génotype 6/6	Genotyp 6/6	F 1444	4

6.4.2 Schematization of the zymogrammes



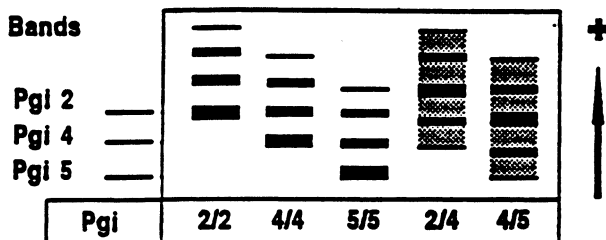
6.5 Recognition of the alleles encoding PGI

6.5.1 Genetic interpretation of the zymogrammes

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

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

6.5.2 Schematization of the zymogrammes



6.6 Recognition of the alleles encoding ACP

6.6.1 Genetic interpretation of the zymogrammes

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

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

EXTRACT FROM TEST GUIDELINES FOR BARLEY (TG/19/10)

Annex, Part II, Pages 3 and 4 of the English Version

Characteristics Derived by Using Electrophoresis

Characteristics Caractères Merkmale	Stage ¹⁾ Stade ¹⁾ Stadium ¹⁾	English	français	deutsch	Example Varieties Exemples Beispielssorten	Note
30. D-Hordein composition: (+) allele expression at locus Hor-3 Composition de l'hordé- ine D: expression de l'allèle occupant le locus Hor-3 D-Hordein-Zusammenset- zung: Allel-Ausprägung in Locus Hor-3		band 34	bande 34	Bande 34	Atem	1
		band 33	bande 33	Bande 33	Natalie	2
		band 35	bande 35	Bande 35	Franka	3
		band 32.5	bande 32,5	Bande 32,5	Iris	4
		band 32	bande 32	Bande 32	Princesse	5
31. C-Hordein composition: (+) allele expression at locus Hor-1 Composition de l'hordé- ine C: expression de l'allèle occupant le locus Hor-1 C-Hordein-Zusammenset- zung: Allel-Ausprägung in Locus Hor-1		bands 62+65+ 68	bandes 62+65+ 68	Banden 62+65+ 68	Atem	1
		bands 62+65+ 66+68	bandes 62+65+ 66+68	Banden 62+65+ 66+68	Regatta	2
		bands 65+68	bandes 65+68	Banden 65+68	Pirate	3
		bands 66.5+71	bandes 66,5+71	Banden 66,5+71	Athos	4
		bands 61.5+ 66.5+71	bandes 61,5+ 66,5+71	Banden 61,5+ 66,5+71	Norka	5
		band 65	bande 65	Bande 65	Birka	6
		bands 60+67.5 +68.5	bandes 60+67,5 +68,5	Banden 60+67,5 +68,5	Pamela	7
		bands 61+65+ 68+73	bandes 61+65+ 68+73	Banden 61+65+ 68+73	Igri	8
		bands 69+72	bandes 69+72	Banden 69+72	Goelette	9
		bands 64+66.5	bandes 64+66,5	Banden 64+66,5	Catinka	10
		bands 67+71	bandes 67+71	Banden 67+71	Ombelle	11
		bands 65+68+ 69+70	bandes 65+68+ 69+70	Banden 65+68+ 69+70	Albacete	12
		bands 61.5+ 68+71	bandes 61,5+ 68+71	Banden 61,5+ 68+71	Borwina	13
		bands 65+67.5	bandes 65+67,5	Banden 65+67,5	Kendo	14
		bands 65.5+ 70.5	bandes 65,5+ 70,5	Banden 65,5+ 70,5	Delita	15
		bands 66+70.5	bandes 66+70,5	Banden 66+70,5	Noveta	16

Characteristics Caractères Merkmale	Stage ¹⁾ Stade ¹⁾ Stadium ¹⁾	English	français	deutsch	Example Varieties Exemples Beispielssorten	Note
32. B-Hordein composition: (+) allele expression at locus Hor-2		bands 79+86+ 88+100	bandes 79+86+ 88+100	Banden 79+86+ 88+100	Atem	1
Composition de l'hordé- ine B: expression de l'allèle occupant le locus Hor-2		bands 79+88+ 91+95+97+101	bandes 79+88+ 91+95+97+101	Banden 79+88+ 91+95+97+101	Aramir	2
		bands 79+91+ 92+95+97+101	bandes 79+91+ 92+95+97+101	Banden 79+91+ 92+95+97+101	Valerie	3
B-Hordein-Zusammenset- zung: Allel-Ausprägung in Locus Hor-2		bands 75+82+ 87+91+97	bandes 75+82+ 87+91+97	Banden 75+82+ 87+91+97	Carina	4
		bands 79+86+ 88+97+101	bandes 79+86+ 88+97+101	Banden 79+86+ 88+97+101	Piroline	5
		bands 78+84+ 95+101	bandes 78+84+ 95+101	Banden 78+84+ 95+101	Catinka	6
		bands 79+90+ 91+94+100	bandes 79+90+ 91+94+100	Banden 79+90+ 91+94+100	Regatta	7
		bands 78+86+ 91+95+100	bandes 78+86+ 91+95+100	Banden 78+86+ 91+95+100	Igri	8
		bands 79+82+ 88+91+92+101	bandes 79+82+ 88+91+92+101	Banden 79+82+ 88+91+92+101	Grit	9
		bands 76+79+ 86+88+100	bandes 76+79+ 86+88+100	Banden 76+79+ 86+88+100	Birka	10
		bands 79+86+ 89+92+95+101	bandes 79+86+ 89+92+95+101	Banden 79+86+ 89+92+95+101	Sigma	11
		bands 79+95+ 101	bandes 79+95+ 101	Banden 79+95+ 101	Midas	12
		bands 78+89+ 92+101	bandes 78+89+ 92+101	Banden 78+89+ 92+101	Criter	13
		bands 75+78+ 79+81+89+101	bandes 75+78+ 79+81+89+101	Banden 75+78+ 79+81+89+101	Ditta	14
		bands 75+78+ 79+81+83+86+ 88+94+95+100	bandes 75+78+ 79+81+83+86+ 88+94+95+100	Banden 75+78+ 79+81+83+86+ 88+94+95+100	Caresse	15
		bands 81+84+ 88+90+101	bandes 81+84+ 88+90+101	Banden 81+84+ 88+90+101	Reseda	16
		bands 75+78+ 79+81+83+86	bandes 75+78+ 79+81+83+86	Banden 75+78+ 79+81+83+86	Baronesse	17
		bands 82+88+ 100	bandes 82+88+ 100	Banden 82+88+ 100	Albacete	18
		bands 81+100	bandes 81+100	Banden 81+100	Digger	19
		bands 75+79+ 83+89+91	bandes 75+79+ 83+89+91	Banden 75+79+ 83+89+91	Camargue	20
		bands 79+91+92	bandes 79+91+92	Banden 79+91+92	Libelle	22
		bands 75+79+ 91+92+95+97+101	bandes 75+79+ 91+92+95+97+101	Banden 75+79+ 91+92+95+97+101	Triton	23
		bands 75+79+ 90+94+99	bandes 75+79+ 90+94+99	bands 75+79+ 90+94+99	Hiberna	24
		bands 79+(83- 85)+(89-91)+ 94-96)+102	bandes 79+(83- 85)+(89-91)+ 94-96)+102	Banden 79+(83- 85)+(89-91)+ 94-96)+102	Jerka	25

Annex, Page 8 of the English Version

Characteristic 31: Locus Hor-1

C-Hordeins

Example variety (Atem)	Note															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
60							--									60
61								--								61
61.5					--							--				61.5
62	--	--	--													62
64										--						64
65	--	--	--	--		--		--			--		--			65
65.5														--		65.5
66		--													--	66
66.5				--	--					--						66.5
67											--					67
67.5								--						--		67.5
68	--	--	--	--				--			--	--				68
68.5								--								68.5
69									--			--				69
70											--	--				70
70.5														--	--	70.5
71				--	--						--		--			71
72									--							72
73									--							73

Characteristic 32: Locus Hor-2

B-Hordeins

Example variety (Atem)	Note																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
75			--											--	--		--		--		--	--				75	
76									--																		76
78					--		--					--	--	--			--										78
79	--	--	--	--	--		--		--	--	--	--	--	--			--		--	--	--	--	--	--	--	--	79
81														--	--	--	--		--								81
82			--					--										--									82
83														--	--			--						--			83
84					--									--	--			--		--				--			84
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91		--	--	--			--	--	--									--		--	--	--				--	91
92		--	--				--	--	--				--							--	--	--					92
94						--								--	--									--	--		94
95		--	--		--		--		--	--				--	--							--	--				95
96																										--	96
97		--	--	--	--																		--				97
99																								--			99
100	--	--			--	--	--	--	--					--	--			--	--								100
101		--	--	--	--		--	--	--	--	--	--	--	--			--	--					--				101
102																									--		102

Annex (Pages 10, 11 and 12 of the English Version)

4.3 Electrophoresis

The acrylic comb is removed from the gel and the sample wells washed with tank buffer (3.2). The tank is filled with an appropriate volume of buffer (3.2) (depending on the equipment used). Samples (10-20 μ l) are loaded into the wells and the gel placed in the tank, ensuring that the sample wells are completely filled. The temperature of the lower buffer chamber should be kept at 15°C. Electrophoresis is carried out at a constant voltage of not more than 60V/cm² (cross-sectional area) of gel (which corresponds to a voltage of 500V for two gels 16 cm wide and 0.15 cm thick) for twice the time taken for the pyronin G marker to leave the gel. It must be remembered that the anode (positive electrode) is at the origin (top of the gel) in this system.

4.4 Fixing and staining

The gel cassette is removed from the tank, opened and the gel placed in a plastic box containing 200 ml of staining solution (3.4.2). Staining is carried out overnight at room temperature. Destaining if necessary is carried out by placing gels in water for about two to 3 hours at room temperature. Gels can then be dried or stored in sealed polythene bags at 4°C.

It should be noted that other procedures, such as the use of increased temperatures or the use of mixtures of TCA and Coomassie Brilliant Blue G, will give satisfactory staining of gels. The final quality control criterion, both for gel preparation and gel staining, is to analyse the suggested example varieties on each batch of gels. The separation of the designated bands, and their relative electrophoretic mobilities, must be clear and correct in order for the procedures to be satisfactory.

States of Expression of the Alleles in the Example Varieties following Acid PAGE

The following Table indicates the REM values of the main bands present in the B- and C-hordein alleles of the example varieties from the Table of Characteristics, following acid PAGE. In comparing the Acid PAGE and SDS PAGE methods, it should be noted that the example varieties and Notes given for the individual states of expression are identical in both methods.

Characteristic	State of Expression	Example Varieties	Note
31. C-hordein composition: (+) allele expression at locus Hor-1	bands 27+30+32+37+39	Atem	1
	bands 27+30+32+34+37+39	Regatta	2
	bands 27+30+32+37	Pirate	3
	bands 32+37+41	Athos	4
	bands 27+30+32+37+39+41	Norka	5
	bands 32+37+38	Birka	6
	bands 35+38	Pamela	7
	bands 32+37+39+41	Igri	8
	bands 38+41+42	Goelette	9
	bands 30+32+37	Catinka	10
	bands 34+37	Ombelle	11
	bands 34+39+41+42	Albacete	12
	bands 31+34+37+38+41	Borwina	13
	bands 32+37+41+43	Kendo	14
	bands 65.5+70.5	Delita	15
bands 66+70.5	Noveta	16	

Characteristic	State of Expression	Example Varieties	Note
32. B-hordein composition: (+) allele expression at locus Hor-2	bands 71+79+83+86+94+100	Atem	1
	bands 71+82+89+100	Aramir	2
	bands 76+82+83+86+100	Valerie	3
	bands 66+71+76+86+93+100	Carina	4
	bands 71+78+79+90+94	Pirolina	5
	bands 76+81+94	Catinka	6
	bands 71+72+75+82+85+86+100	Regatta	7
	bands 72+76+79+90+94	Igri	8
	bands 71+76+79+86	Grit	9
	bands 71+78+83+86+94+100	Birka	10
	bands 71+79+83+86+90	Sigma	11
	bands 71+76+79	Midas	12
	bands 71+89	Criter	13
	bands 79+83+86+90	Ditta	14
	bands 67+69+71+72+78+79+85+89+94	Caresse	15
	bands 71+79+83+88+94	Reseda	16
	bands 69+76+79+83+93	Baronesse	17
	bands 71+72+79+85+86+91+100	Albacete	18
	bands 72+76+100	Digger	19
	bands 61+71+76+79+83	Camargue	20
	bands 76+81+94+100	Marko	21
	bands 79+91+92	Libelle	22
	bands 75+79+91+92+95+97+101	Triton	23
	bands 75+79+90+94+99	Hiberna	24
	bands 79+ (83-85) + (89-91) + (94-96) +102	Jerka	25

Recognition of Hordein Alleles

B- and C-Hordeins: nomenclature of the individual bands and recognition of the corresponding alleles: acid PAGE

Example variety (Atem)	C-Hordeins																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
25																	25
27	--	--	--	--	--												27
30	--	--	--	--	--					--					--		30
31													--				31
32	--	--	--	--	--	--		--		--				--			32
34		--									--	--	--				34
35							--										35
37	--	--	--	--	--	--		--		--	--		--	--	--	--	37
38						--	--		--				--				38
39	--	--	--		--		--					--			--		39
41				--	--			--	--			--	--	--			41
42									--			--				--	42
43														--			43
	10	10A	1	11	17	6	19	2	4	5	18	14	8	3	15	7	

Alleles according to acid PAGE nomenclature.

B-Hordeins

Example variety (Atem)	Note																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
61																				--						61
66				--																						66
67															--											67
69															--		--									68
71	--	--	--	--	--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	71
72							--	--							--			--	--	--				--	--	72
75							--																	--	--	75
76			--	--		--		--	--		--						--		--	--	--	--	--	--	--	76
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79	--	--			--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	79
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83	--	--								--	--			--	--	--	--	--	--	--	--	--	--	--	--	83
85							--							--	--			--						--	--	85
86	--	--	--	--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	86
88															--											88
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90					--		--			--			--													90
91																		--								91
93				--													--									93
94	--	--		--	--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	94
97																									--	97
100	--	--	--	--	--		--			--								--	--	--	--	--	--	--	--	100
104		3	4	13	14	-	9	1	7	6	-	-	11	16	-	18	-	19	8	15	12	10	13	13	1	32

Alleles according to acid PAGE nomenclature.

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