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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS

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WORKING PAPER ON REVISED TEST GUIDELINES FOR POTATO

Document prepared by experts from Germany

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I. Subject of these Guidelines

These Test Guidelines apply to all varieties of *Solanum tuberosum* L.

II. Material Required

1. The competent authorities decide when, where and in what quantity and quality the plant material required for testing the variety is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must make sure that all customs formalities are complied with. The minimum quantity of plant material should be:

150 tubers in each year of testing

The diameter of the tubers to be delivered should be 35 to 50 mm. The plant material supplied should be visibly healthy, not lacking in vigor or affected by any important pest or disease.

2. The plant material should not have undergone any treatment unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

III. Conduct of Tests

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

2. The tests should normally be conducted at one place. If any important characteristics of the variety cannot be seen at that place, the variety may be tested at an additional place.

3. The field tests should be carried out under conditions ensuring normal growth. The size of the plots should 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 period. As a minimum, each test should include a total of 60 plants which should be divided between two or more replicates. Separate plots for observation and for measuring can only be used if they have been subject to similar environmental conditions.
The lightsprout test should include a total of 8 tubers as a minimum.

4. Additional tests for special purposes may be established.

IV. Methods and Observations

1. All observations should be made on the total number of plants if not otherwise indicated.

2. For the assessment of uniformity a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 60 plants, the maximum number of off-types allowed would be 2.

V. Grouping of Varieties

1. The collection of varieties to be grown should 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 are fairly evenly distributed within the collection.

2. It is recommended that the competent authorities use the following characteristics for grouping varieties:

Lightsprout: color of anthocyanin coloration of base (characteristic 4)
Flower corolla: color of inner side (characteristic 33)
Flower corolla: color of inner side in colored flower (characteristic 34)
Time of maturity (characteristic 37)
Tuber: color of skin (characteristic 40)

VI. Characteristics and Symbols

1. To assess distinctness, uniformity and stability, the characteristics and their states as given in the Table of Characteristics should be used.

2. Notes (1-9), for ease of handling, are given opposite the states of the different characteristics.

Legend:

(*) Characteristics which should be used every growing period for the examination of all varieties and should always be included in the description of the variety, except when the state of expression of a preceding characteristic or regional environmental conditions render this impossible.

(+) See Explanations on the Table of Characteristics in chapter VIII.

[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 at the end of chapter VIII.

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

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

	Stage [1] Stade [1] Stadium [1] Estado [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1 (+)	**1 VG**	**Lightsprout: size**					
		small					3
		medium					5
		large					7
2. (*) (+)	**1 VG**	**Lightsprout: shape**					
		spherical					1
		ovoid					2
		conical					3
		broad cylindrical					4
		narrow cylindrical					5
3. (*) (+)	**1 VG**	**Lightsprout: color of base**					
		green					1
		anthocyanin colored					2
4. (*) (+)	**1 VG**	**Lightsprout: color of anthocyanin coloration of base**					
		red-violet					1
		violet					2
		blue-violet					3

	Stage [1] Stade [1] Stadium [1] Estado [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
5. (*) (+)	**1 VG**	**Lightsprout: intensity of anthocyanin coloration of base**					
		very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
6. (+)	**1 VG**	**Lightsprout: pubescence of base**					
		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
7. (+)	**1 VG**	**Lightsprout: size of tip in relation to base**					
		very small					1
		small					3
		medium					5
		large					7
		very large					9

	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
8. **(+)**	**1 VG**	**Lightsprout: habit of tip**					
		closed					1
		half open					3
		open					5
9. **(+)**	**1 VG**	**Lightsprout: anthocyanin coloration of tip**					
		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
10. **(+)**	**1 VG**	**Lightsprout: pubescence of tip**					
		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
11. **(*)** **(+)**	**1 VG**	**Lightsprout: number of root tips**					
		few					3
		medium					5
		many					7

	Stage [1] / Stade [1] / Stadium [1] / Estado [1]	English	français	deutsch	español	Example Varieties / Exemples / Beispielssorten / Variedades ejemplo	Note/ Nota
12. **(+)**	**1 VG**	**Lightsprout: length of lateral shoots**					
		absent or very short					1
		short					3
		medium					5
		long					7
		very long					9
13. **(+)**	**2 VG**	**Plant: Type**					
		stem type					1
		intermediate type					2
		leaf type					3
14. **(*)** **(+)**	**2 VG**	**Plant: growth habit**					
		erect					1
		semi-erect					3
		spreading					5
15. **(*)**	**2 VG**	**Stem: spreading of anthocyanin coloration**					
		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9

	Stage [1] Stade [1] Stadium [1] Estado [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
16. **(+)**	**2 VG**	**Leaf: outline size**					
		very small					1
		small					3
		medium					5
		large					7
		very large					9
17. **(+)**	**2 VG**	**Leaf: silhouette**					
		closed					1
		half open					3
		open					5
18.	**2 VG**	**Leaf: green color**					
		light					3
		medium					5
		dark					7
19. **(+)**	**2 VG**	**Leaf: spreading of anthocyanin coloration of midrib on upper side**					
		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9

	Stage [1] Stade [1] Stadium [1] Estado [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
20. **(+)**	**2 VG**	**<u>Terminal</u> leaflet: width in relation to length**					
		narrow					3
		medium					5
		broad					7
21. **(+)**	**2 VG**	**<u>Terminal</u> leaflet: frequency of coalescence**					
		absent or very low					1
		low					3
		medium					5
		high					7
		very high					9
22.	**2 VG**	**Leaflets: waviness of margin**					
		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
23.	**2 VG**	**Leaflets: color of apical rosette**					
		anthocyanin colored					1
		yellow green					2
		medium green					3
		dark green					4

	Stage [1] Stade [1] Stadium [1] Estado [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
24.	**2 VG**	**Leaflets: pubescence of blade at apical rosette**		Proposed by RU. No experience with this char. in DE and NL.			
		absent					1
		present					9
25.	**2 VG**	**Leaflets: glossiness of the upperside**					
		dull					3
		medium					5
		glossy					7
26. (+)	**2 VG**	**Leaf: intensity of secondary structure**					
		weak					3
		medium					5
		strong					7
27.	**2 VG**	**Flower bud: spreading of anthocyanin coloration**					
		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9

	Stage [1] Stade [1] Stadium [1] Estado [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
28.	**3 VG**	**Plant: height**					
		very short					1
		short					3
		medium					5
		tall					7
		very tall					9
29. (*)	**3 VG**	**Plant: frequency of flowers**					
		absent or very low					1
		low					3
		medium					5
		high					7
		very high					9
30. (+)	**3 VG**	**Inflorescence: size**					
		very small					1
		small					3
		medium					5
		large					7
		very large					9

	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
31. **(+)**	**3** **VG**	**Inflorescence: spreading of anthocyanin coloration of peduncle**					
		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
32. **(+)**	**3** **VG**	**Flower corolla: size**					
		very small					1
		small					3
		medium					5
		large					7
		very large					9
33. **(*)**	**3** **VG**	**Flower: corolla: color of inner side**					
		white					1
		anthocyanin colored					2
34. **(*)**	**3** **VG**	**Flower: corolla: color of inner side in colored flower**					
		red-violet					1
		violet					2
		blue-violet					3

	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
35. (*)	**3 VG**	**Flower corolla: intensity of anthocyanin coloration of inner side**					
		very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9
36. (*) (+)	**3 VG**	**Flower corolla: size of white tips in colored flower**					
		absent or very small					1
		small					3
		medium					5
		large					7
		very large					9
37. (*)	**4 VG**	**Plant: time of maturity**					
		very early					1
		early					3
		medium					5
		late					7
		very late					9

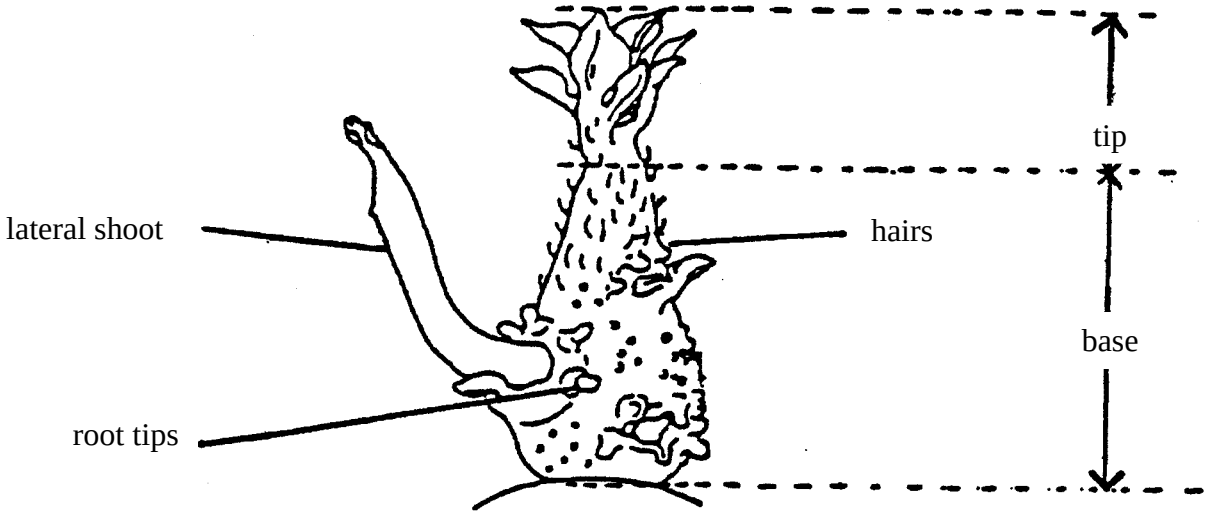
	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
38. (*) (+)	**5 MS**	**Tuber: shape**					
		round					1
		short-oval					2
		oval					3
		long-oval					4
		long					5
		very long					6
39.	**5 VG**	**Tuber: depth of eyes**					
		very shallow					1
		shallow					3
		medium					5
		deep					7
		very deep					9
40. (*)	**5 VG**	**Tuber: color of skin**					
		yellow					1
		red					2
		blue					3
		red parti-colored					4
		blue parti-colored					5
41.	**5 VG**	**Tuber: intensity of color of skin**					
		light					3
		medium					5
		dark					7

	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
42. **(*)**	**5** **VG**	**Tuber: color of base of eye**					
		yellow					1
		red					2
		blue					3
43. **(*)**	**5** **VG**	**Tuber: color of flesh**					
		white					1
		cream					2
		light yellow					3
		medium yellow					4
		dark yellow					5
		blue					6
		red parti-colored					7
44. **(+)**	**5** **VG**	**<u>Yellow skinned varieties only:</u>** **Tuber: antho-cyanin coloration of skin in reaction to light**					
		absent or very weak					1
		weak					3
		medium					5
		strong					7
		very strong					9

VIII. Explanations on the Table of Characteristics

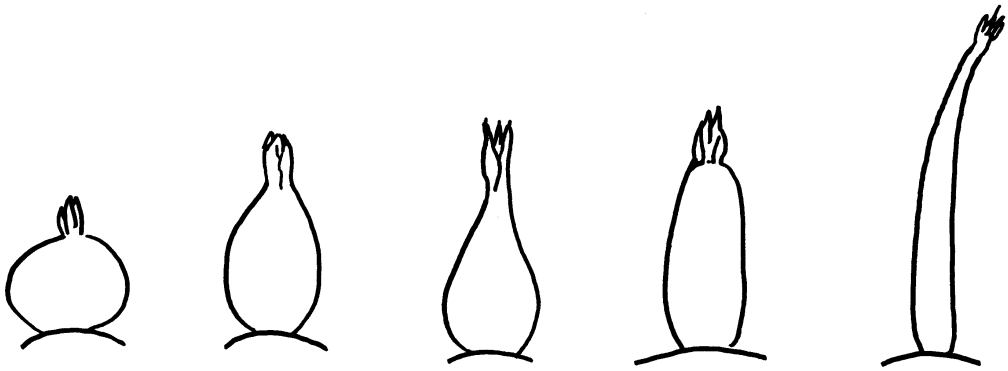
Ads. 1-12: Lightsprout

Lightsprout



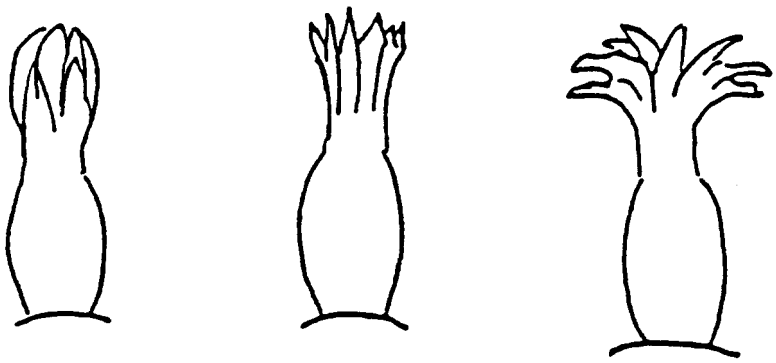
The spectrum and the intensity of the light source are the most determining factors for the expression of characteristics of lightsprouts. This spectrum is unambiguously defined by the type of lamps and the voltage used. Day light should be excluded. For illumination standardized fluorescent tubes e.g. “Osram L 58W/77 Fluora” and “Philipps TLD 58W/29 Warmtone” in combination, should be used for 5 hours per day. The lamps each with one tube of both types are located in front of the shelv units in a distance of 60 cm to the tubers and 1 m to each other. Alternatively, continuous light of small incandescent bulbs (6V AC/0,05 A), 8 per square meter, 15-25 cm above the tubers can be used.

Ad. 2: Lightsprout: Shape



1	2	3	4	5
spherical	ovoid	conical	broad cylindrical	narrow cylindrical

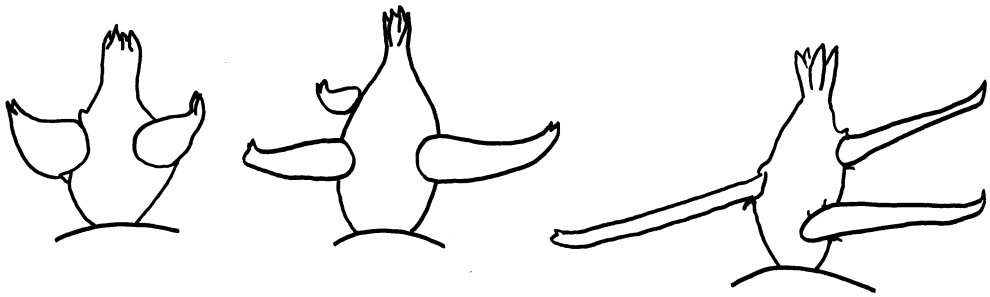
Ad. 8: Lightsprout: habit of tip



1	3	5
closed	half open	open

The characteristic should be observed after about 10 weeks when a good differentiation in the collection is reached.

Ad. 12: Lightsprout: length of lateral shoots



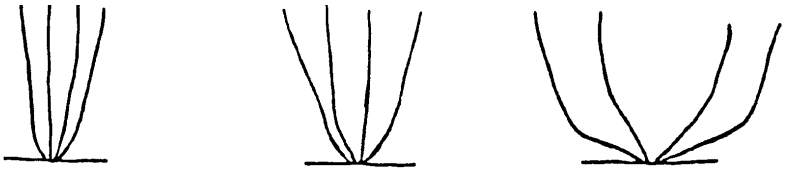
3	5	7
short	medium	long

Ad. 13: Plant: type

Stem-type: foliage open, stems clearly visible
Intermediate: foliage half open, stems partly visible
Leaf-type: foliage closed, stems not or hardly visible

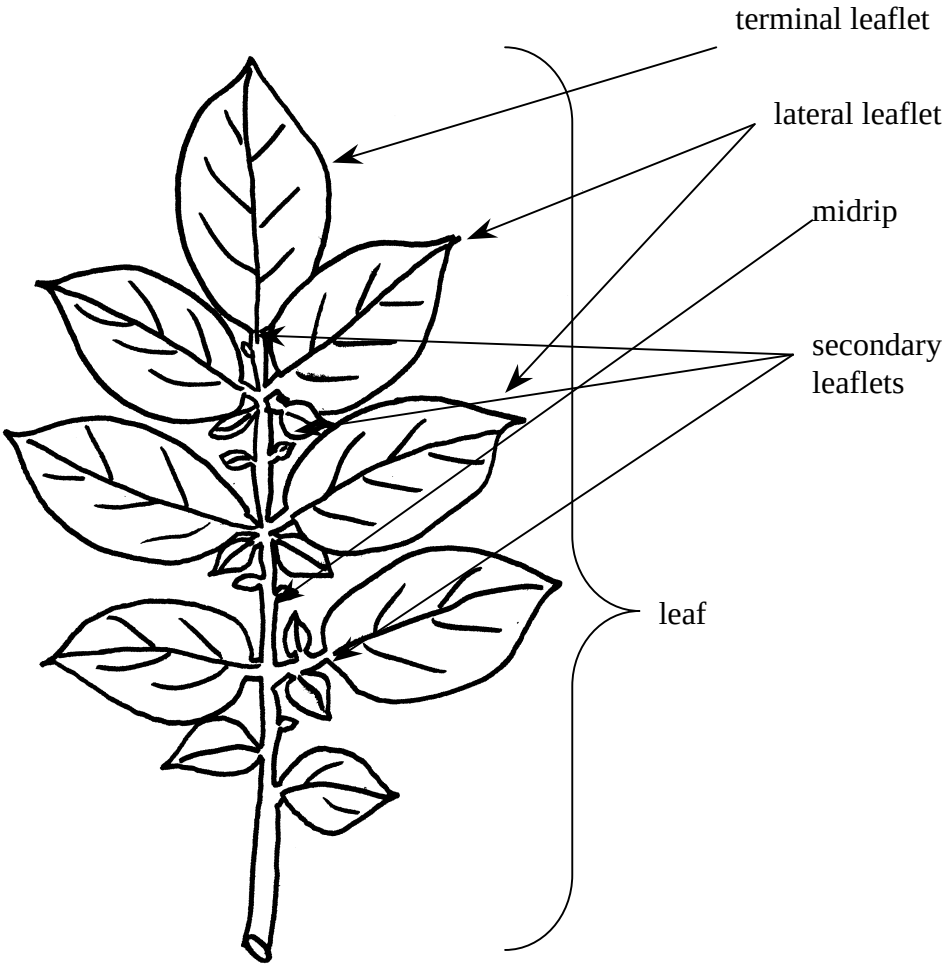
1	3	5
stem-type	intermediate-type	leaf-type

Ad. 14: Plant: growth habit

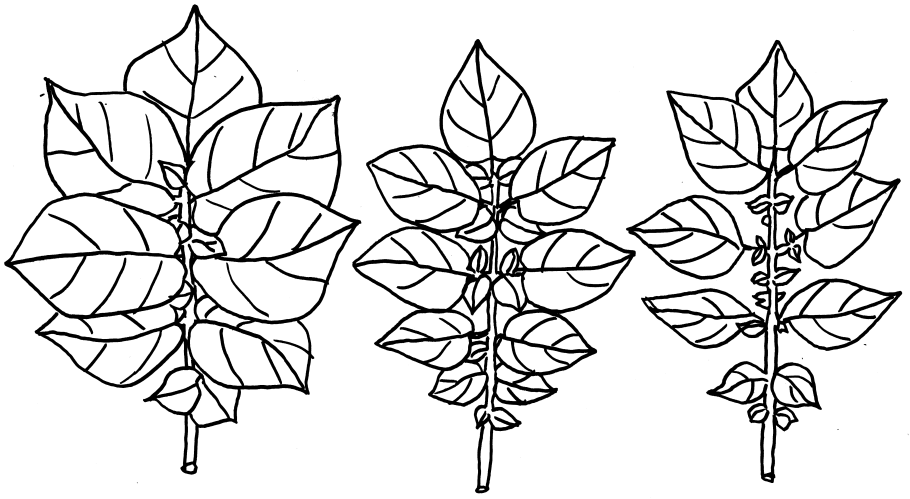


1	3	5
erect	semi-erect	spreading

Ads. 16 to 26: Leaf characteristics



Ad. 17: Leaf: silhouette

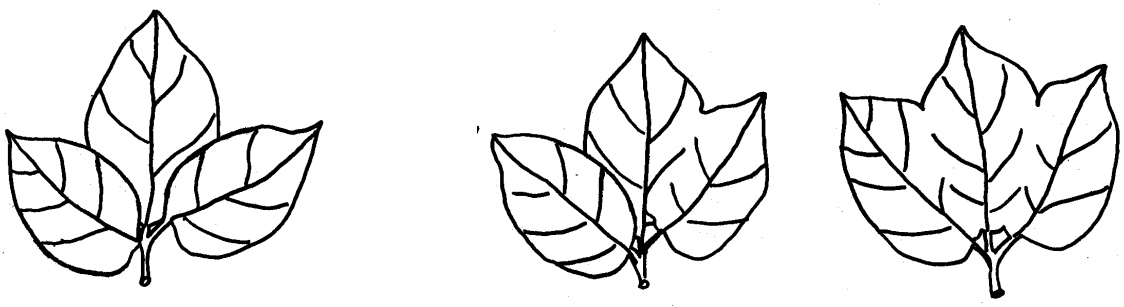


1	3	5
closed	half open	open

Ad. 20: Terminal leaflet: width in relation to length

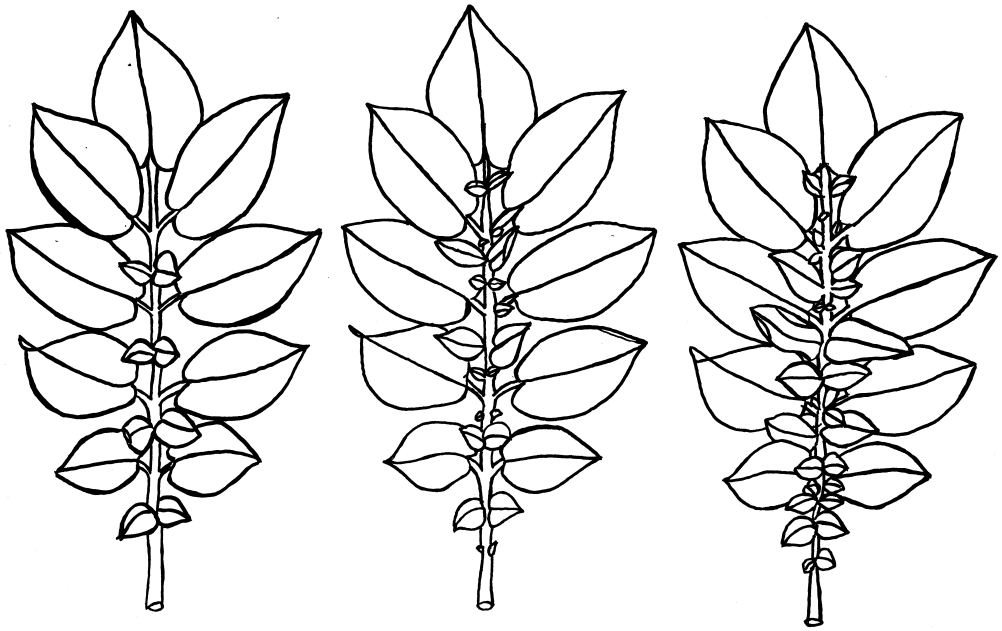
3	5	7
narrow	medium	broad

Ad. 21: Terminal leaflet: frequency of coalescence



not coalescent coalescent

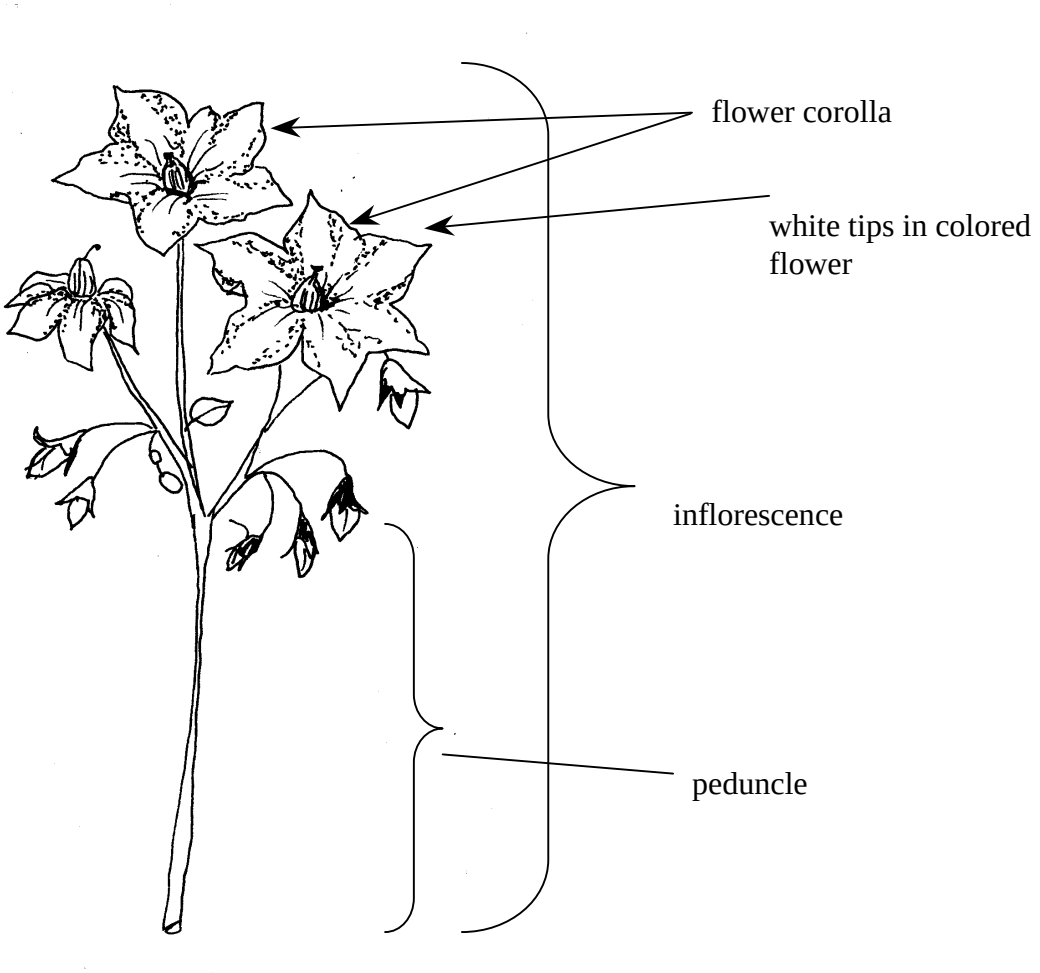
Ad. 26: Leaf: intensity of secondary structure



3 5 7

weak medium strong

Ad. 30–36: Flower characteristics



Ad. 38: Tuber: shape (100 x length/width)

1	round	≤ 109
2	short-oval	110 – 129
3	oval	130 – 149
4	long-oval	150 – 169
5	long	170 – 199
6	very long	≥ 200

Ad. 44: Yellow skinned varieties only: Tuber: anthocyanin coloration of skin in reaction to light

The anthocyanin development in the skin of yellow skinned varieties should be assessed after 10 days of exposure to full daylight or after 150 hours of exposure to artificial light.

OPTIMAL STAGE OF ASSESSMENT OF CHARACTERISTICS

1 = about 12 weeks after starting

2 = bud stage

3 = flowering stage

4 = ripening stage

5 = after harvest

IX Literature

X. Technical Questionnaire

	Reference Number (not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE

To be completed in connection with an application for plant breeders' rights

1. Species	*Solanum tuberosum* L. POTATO
2. Applicant (Name and address)	
3. Proposed denomination or breeder's reference	

4. Information on origin, maintenance and reproduction of the variety

5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the state of expression which best corresponds).

	Characteristics	Example Varieties	Note
5.1 (3)	**Lightsprout: color of base**		
	green		1[ ]
	anthocyanin colored		2[ ]
5.2 (4)	**Lightsprout: color of anthocyanin coloration of base**		
	red-violet		1[ ]
	violet		2[ ]
	blue-violet		3[ ]
5.3 (29)	**Plant: frequency of flowers**		
	absent or very low		1[ ]
	low		3[ ]
	medium		5[ ]
	high		7[ ]
	very high		9[ ]
5.4 (33)	**Flower: corolla: color of inner side**		
	white		1[ ]
	anthocyanin colored		2[ ]

	Characteristics	Example Varieties	Note
5.5 (34)	**Flower corolla; color of inner side in colored flower**		
	red-violet		1[ ]
	violet		2[ ]
	blue-violet		3[ ]
5.6 (37)	**Plant: time of maturity**		
	very early		1[ ]
	early		3[ ]
	medium		5[ ]
	late		7[ ]
	very late		9[ ]
5.7 (38)	**Tuber: shape**		
	round		1[ ]
	short-oval		2[ ]
	oval		3[ ]
	long-oval		4[ ]
	long		5[ ]
	very long		6[ ]
5.8 (40)	**Tuber: color of skin**		
	yellow		1[ ]
	red		2[ ]
	blue		3[ ]
	red parti-colored		4[ ]
	blue parti-colored		5[ ]

	Characteristics	Example Varieties	Note
5.9 (42)	**Tuber: color of base of eye**		
	yellow		1[ ]
	red		2[ ]
	blue		3[ ]
5.10 (43)	**Tuber: color of flesh**		
	white		1[ ]
	cream		2[ ]
	light yellow		3[ ]
	medium yellow		4[ ]
	dark yellow		5[ ]
	blue		6[ ]
	red parti-colored		7[ ]

6. Similar varieties and differences from these varieties

Denomination of similar variety	Characteristic in which the similar variety is different o)	State of expression of similar variety	State of expression of candidate variety

o) In the case of identical states of expressions of both varieties, please indicate the size of the difference.

7. Additional information which may help to distinguish the variety

7.1 Resistance to pests and diseases

7.2 Special conditions for the examination of the variety

7.3 Other information

8. Authorization for release

(a) Does the variety require prior authorization for the 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 that question is yes, please attach a copy of such an authorization.

[Annex follows]

ANNEX

ADDITIONAL USEFUL EXPLANATIONS

Table of Contents

PART I

INTRODUCTION

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

PART II

CHARACTERISTICS DERIVED BY USING ELECTROPHORESIS

Stage [1] Stade [1] Stadium [1] Estado [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
44.	Allele expression at loci Est 2 and Est 3					
	Genotype j+o				Hansa	1
	Genotype l+c				Sieglinde	2
	Genotype j+c				Karolin	3
	Genotype a+o				Desiree	4
	Genotype d+o				Achat	5
	Genotype h+o				Jetta	6
	Genotype i+b				Selma	7
	Genotype i+o				Renate	8
	Genotype j+b				Ute	9
	Genotype o+o				Ulla	11
	Genotype f+o				Walli	12
	Genotype k+o				Belita	13
	Genotype i+c				Karakter	15
	Genotype l+o				Roxy	16
	Genotype k+d				Junior	17
	Genotype b+o				Cleopatra	18
	Genotype d+c				Krometa	19
	Genotype e+o				Sibu	20
	Genotype c+o				Obelix	22
	Genotype d+b				Vital	23
	Genotype g+b				Premiere	26
	Genotype jf+o				Protea	27

Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
45.	**Allele expression at locus Prx**					
	Genotype a or j				Hansa	1
	Genotype b				Corine	2
	Genotype c				Tomensa	3
	Genotype d				Amigo	4
	Genotype e				Jetta	5
	Genotype g				Thomana	6
	Genotype f				Diana	7
	Genotype h				Kanjer	8
46.	**Allele expression at locus Pat-**					
	Genotype 9.01				Calla	1
	Genotype 6.01				Artana	2
	Genotype 7.06				Karnico	4
	Genotype 1.01				Secura	6
	Genotype 6.02				Quinta	7
	Genotype 2.01				Erntestolz	9
	Genotype 2.02				Desiree	11
	Genotype 5.01				Belita	13
	Genotype 5.02				Solina	14
	Genotype 2.04				Delia	16
	Genotype 7.01				Fausta	17
	Genotype 3.01				Quarta	19
	Genotype 7.04				Grata	20
	Genotype 3.02				Irmgard	21
	Genotype 7.05				Atica	23
	Genotype 7.03				Pallina	25

Stage [1] Stade [1] Stadium [1] Estado [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
46. (cont.)	**Allele expression at locus Pat (cont.)**					
	Genotype 3.08				Danva	26
	Genotype 8.06				Padea	28
	Genotype 8.10				Karida	29
	Genotype 8.07				Elles	30
	Genotype 4.01				Indira	31
	Genotype 8.03				Darwina	33
	Genotype 4.02				Christa	34
	Genotype 8.02				Escort	35
	Genotype 8.14				Sirius	36
	Genotype 8.13				Krometa	37
	Genotype 8.12				Arnika	39
	Genotype 4.08				Sommergold	40
	Genotype 4.12				Saturna	42
	Genotype 4.07				Cinja	43
	Genotype 8.11				Vebeca	44
	Genotype 4.11				Pepo	45
	Genotype 3.03				Ulla	47
	Genotype 3.04				Fasan	49
	Genotype 3.09				Combi	50
	Genotype 7.07				Franca	51
	Genotype 3.05				Karolin	52
	Genotype 4.04				Rubin	53
	Genotype 4.03				Pia	54
	Genotype 8.04				Shepody	55
	Genotype 4.09				Walli	57

Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
46. (cont.)	**Allele expression at locus Pat (cont.)**					
	Genotype 3.07				Junior	58
	Genotype 7.08				Adretta	60
	Genotype 3.06				Gloria	61
	Genotype 7.11				Ukama	62
	Genotype 10.01				Liu	63
	Genotype 4.05				Cleopatra	65
	Genotype 4.06				Felsina	67
	Genotype 8.05				Kardal	68
	Genotype 8.15				Albas	70
	Genotype 8.16				Feska	72
	Genotype 4.14				Aiko	73
	Genotype 8.08				Solara	74
	Genotype 4.15				Amigo	75
	Genotype 8.09				Thomana	76
	Genotype 2.03				Pompadur	77
	Genotype 10.02				Kranich	80
	Genotype 4.16				Möwe	85
	Genotype 7.02				Orlando	86
	Genotype 4.17				Oktan	87

PART III

DESCRIPTION OF THE METHODS TO BE USED

Polyacrylamide gel electrophoresis methods for the analysis of esterases, peroxydases and patanins in potatoes

1. Number of tubers per test

- for distinctness, uniformity and stability: 10 tubers
- for checking identity: 4 tubers

The tubers should be mature, preferably harvested after senescence of foliage. Tubers stored between 4 -10° C can be used independent of the season as long as there is no or only slight sprouting.

2. Apparatus and equipment

Centrifuge
Cryostat
Power supply with a capacity of at least 400 V and 150 mA
Rocking platform shaker
Vertical dual slab gel system

Any suitable vertical electrophoresis system can be used, provided that the gels can be kept at a constant temperature. A gel thickness of no more than 1.5 mm is recommended. The power supply should be capable of delivering both constant current and constant voltage output.

3. Chemicals

All chemicals should be of “Analytical Reagent” grade or better.

3.1. Chemicals for protein extraction

Amidoblack 10 B
Sodium disulphite $Na_2S_2O_5$
Sodium sulphite Na_2SO_3
Sucrose

3.2. Chemicals for electrophoresis

40% Acrylamide solution **(Security advice: Acrylamide is an extremely toxic chemical!)**
Ammonium persulfate (APS)
2% Bisacrylamide solution
Boric acid
Bromophenol blue (BPB)
3-(Dimethylamino)propionitrile (DMAPN)
Ethanol
Glycine
Hydrochloric acid (HCl)
Sucrose
NNN´N`-Tetramethylethylenediamine (TEMED)
Tris-(hydroxymethyl)-aminomethane (TRIS)

3.3. Chemicals for staining of proteins

Acetone
Coomassie Blue G250
Coomassie Blue R250
Dianisidine-2HCl **(Security advice: Dianisidine is an extremely toxic chemical !)**
Disodium hydrogen phosphate -Dodecahydrate (Na_2HPO_4 x $12H_2O$)
Fast Blue RR Salt
Glacial acetic acid
Glycerol
30% Hydrogen peroxyde
Methanol
1-Naphthyl acetate
Sodium dihydrogen phosphate - Monohydrate (NaH_2PO_4 x $1H_2O$)
Trichloroacetic acid (TCA)

4. Solutions

4.1. Extraction solutions

No.	Solution	Ingredients	Amount	Remark
4.1.1.	Extraction solution A	Sodium sulphite Sodium disulphite de-ionised water	5.00 g 3.75 g 100 ml	to be stored at 6° C
4.1.2.	Extraction solution B	Sucrose Amidoblack 10B de-ionised water	500 g 0.3 g ad 1000 ml	to be stored at 6° C
4.1.3.	Extraction solution C	Extraction solution A Extraction solution B	10 ml 100 ml	to be prepared daily

4.2. Electrophoresis buffers and gel preparation solutions

4.2.1. Buffers and Solutions for PAGE pH 7.9 of the esterases

No.	Solution	Ingredients	Amount	Remark
4.2.1.1.	Stock gel buffer	TRIS Boric acid de-ionised water	30.26 g 36.60 g ad 1000 ml	
4.2.1.2.	40% Acrylamide solution	Acrylamide de-ionised water	40 g ad 100 ml	For safety a commercial solution should be used
4.2.1.3.	2% BIS solution	Bis acrylamide de-ionised water	2 g ad 100 ml	For safety a commercial solution should be used
4.2.1.4.	2% APS solution	Ammonium persulfate de-ionised water	1 g ad 50 ml	to be prepared daily
4.2.1.5.	Tank buffer	Stock gel buffer 4.2.1.1. de-ionised water	125 ml 875 ml	to be prepared daily

4.2.2. Buffers and Solutions for PAGE pH 8.9 of the peroxydases and patanins

No.	*Solution*	Ingredients	Amount	Remark
4.2.2.1	Resolving gel buffer	TRIS de-ionised water	75.4 g ad 1000 ml	adjusted to pH 8.9 with HCl.
4.2.2.2	Stacking gel buffer	TRIS Bromophenol blue de-ionised water	16 g 100 mg ad 1000 ml	adjusted to pH 6.7 with HCl
4.2.2.3.	Stacking gel preparing solution	Stacking gel buffer 4.2.2.2. 40% Acrylamide solution 2% Bisacrylamide solution de-ionised water Sucrose	280 ml 45 ml 73 ml 150 ml 80 g	
4.2.2.4	40% Acrylamide solution	Acrylamide de-ionised water	40 g ad 100 ml	For safety a commercial solution should be used
4.2.2.5.	2% BIS solution	Bis acrylamide de-ionised water	2 g ad 100 ml	For safety a commercial solution should be used
4.2.2.6.	2% APS solution	Ammonium persulfate de-ionised water	0.4 g ad 20 ml	to be prepared daily
4.2.2.7.	10% Ethanol solution	Ethanol de-ionised water	10 ml ad 100 ml	
4.2.2.8.	Stock tank buffer	TRIS Glycine de-ionised water	5.2 mg 3.5 g ad 1000 ml	
4.2.2.9.	Tank buffer	Stock tank buffer (4.2.2.8.) de-ionised water	50 ml ad 1000 ml	to be prepared daily

4.3. Staining Solutions for patanins, peroxydases and esterases

No.	*Solution*	Ingredients	Amount	Remark
4.3.1.	Stock solution	Coomassie Blue G 250 Coomassie Blue R 250 de-ionised water	0.25 g 0.75 g ad 100 ml	to be stirred for at least 1 h; to be shaken very well before use
4.3.2.	Staining solution for patanins	TCA Glacial acetic acid normal water Methanol Stock solution (4.3.1.)	240 g 280 ml 3300 ml 600 ml 100 ml	
4.3.3.	Staining buffer A for esterases	Na_2HPO_4 x $12H_2O$ de-ionised water	53.7 g ad 1000 ml	
4.3.4.	Staining buffer B for esterases Staining buffer for peroxydases	NaH_2PO_4 x $1H_2O$ de-ionised water	20.7 g ad 1000 ml	
4.3.5.	Dianisidine solution	Dianisidine-2 HCl de-ionised water	1 g ad 100 ml	can be stored at 6° C for 1 week
4.3.6.	2% Glyceriol solution	Glycerol water	20 g ad 1000 ml	

5. Procedure

5.1 Preparation of the sample

The tubers are frozen in a deepfreezer at –20° C and then thawed at room temperature.

A 2 ml screw topped tube containing 0.4 ml extraction solution C (4.1.2.) is needed for the analysis of each tuber.

The thawed tubers are cut in two halves and squeezed out. 1.5 ml of the sap are collected in the above mentioned tube and mixed with the extraction solution C by shaking. Now the solutions are centrifugated for 15 min at 3000 Rpm and 10° C. The clear supernants are transferred into new, empty 2-ml-screw topped tubes and are then frozen. Before starting the electrophoresis the protein extracts are thawed and transferred as aliquots of 0.15 ml in a microtiter plate.

5.2 Preparation of the gels

5.2.1. Preparation of the gels for PAGE pH 7.9 of the esterases

Clean and dry gel cassettes are assembled, according to the design of the equipment used.

Preparation of about 100 ml gel solution (T: 4.9%; C: 4.7%):

Under carefully stirring 108 mg sodium sulphite are dissolved in 55 ml de-ionised water. The following solutions are added:

30 ml Stock gel buffer (4.2.1.1.),
30 ml 40% Acrylamide solution (4.2.1.2.) and
30 ml 2% BIS solution (4.2.1.3.).

Finally the polymerisation is started by addition of
1.2 ml DMAPN solution and
4.5 ml 2% APS solution (4.2.1.4.).

After mixing the gels are carefully poured, avoiding the formation of air bubbles. The well-forming "combs" are inserted in the liquid gels and the polymerisation is allowed to take place at room temperature for at least 15 min. The "combs" are then removed carefully from the gel cassettes. The wells are rinsed using tank buffer (4.2.1.5.).

5.2.2. Preparation of the gels for PAGE pH 8.9 of the peroxydases and patanins

Clean and dry gel cassettes are assembled, according to the design of the equipment used.

Each gel consists of resolving gel and stacking gel.

Preparation of about 100 ml resolving gel solution (T: 5.5%; C: 4.4%):

The following solutions are mixed under slowly stirring:

60 ml resolving gel buffer (4.2.2.1.),
14 ml de-ionised water,
14 ml 40% Acrylamide solution (4.2.2.4.) and
13 ml 2% BIS solution 4.2.2.5.).

Finally the polymersation is started by addition of
100 µl TEMED and
6 ml 2% APS solution (4.2.2.6.)

The gels are carefully poured, avoiding the formation of air bubbles, and the polymerisation is allowed to take place at room temperature for at least 15 min. The gel cassettes should not be filled entirely, in order to leave room for a 14 mm layer of stacking gel. The gel surface is carefully overlapped with 10% ethanol solution (4.2.2.7.) using a syringe. When the polymerisation is finished, the gel surface is rinsed with de-ionised water and dried with filter paper.

Preparation of stacking gels:

15 ml stacking gel buffer (4.2.2.3.),
60 µl TEMED and
375 µl 2 % APS-Lösung (4.2.2.6.) are mixed under slowly stirring.

The gels are carefully poured, avoiding the formation of air bubbles. The well-forming "combs" are inserted in the liquid gels and the polymerisation is allowed to take place at room temperature for around 15 min. The "combs" are then removed carefully from the gel cassettes. The wells are rinsed using tank buffer (4.2.2.9.).

5.3 Sample loading

For the electrophoretic separation of the esterases and peroxydases each gel well is filled with 6 µl - 12 µl extract from microtiter plate (see 5.1.) depending on the size of the comb well. For the electrophoretic separation of the patanins each gel well is filled with 3 µl - 6 µl extract from microtiter plate (see 5.1.) depending on the size of the comb well.

5.4 Electrophoresis

5.4.1. Conditions for PAGE pH 7.9 of the esterases

Tank buffer	= Solution 4.2.1.5
Current for a gel (11 cm broad, 1 mm thick)	= in the beginning 40 mA, and then 80 mA
Voltage	= max. 300 V
Temperature	= 5° C to 15° C
Migration way	= from the cathode (-) to the anode (+)
Migration distance	= 6 cm Amido black

5.4.2. Conditions for PAGE pH 8.9 of the peroxydases and patanins

Tank buffer	= Solution 4.2.2.9.
Current for a gel (11 cm broad, 1 mm thick)	= in the beginning 40 mA, and then 80 mA
Voltage	= max. 300 V
Temperature	= 5° C to 15° C
Migration way	= from the cathode (-) to the anode (+)
Migration distance	= 6 cm Bromphenol blue

5.5 Staining

5.5.1. Staining of esterases

Gels from the PAGE pH 8.9 are marked, e.g. by cutting the gels corner. Then the gels are transferred in a staining container filled with a mixture of 120 ml staining buffer A (4.3.3.) and 80 ml staining buffer B (4.3.4.) and incubated on a rocking platform shaker. 50 mg 1-Naphthylacetate are dissolved in 3 drops acetone and diluted with des-ionised water, until this solution becomes turbid. The solution is added to the buffer solution with the gels. 100 mg Fast blue RR salt are suspended in 5 ml acetone and diluted with 5 ml des-ionosed water. This solution is added to the buffer solution with the gels immediately.

The staining time ranges between 15 and 40 minutes. For destaining the gels are incubated on the shaker in des-ionised water for 2 x 30 min. Finally the gels are incubated on

the shaker in 2% glycerol solution (4.3.6.) for 30 min. After this incubation the gels are dried between two layers of cellophane soaked in 2% glycerol solution (4.3.6.).

5.5.2. Staining of peroxydases

Gels from the PAGE pH 8.9 are marked, e.g. by cutting the gels corner. Then the gels are transferred in a staining container filled with 200 ml staining buffer (4.3.4.) and incubated on a rocking platform shaker. 10 ml Dianisidine solution (4.3.5.) are added. After 30 sec the staining reaction is started by addition of 260 µl 30% hydrogen peroxyde.

The staining time ranges between 10 and 20 minutes. For destaining the gels are incubated on the shaker in des-ionised water for 2 x 30 min. Finally the gels are incubated on the shaker in 2% glycerol solution (4.3.6.) for 30 min. After this incubation the gels are dried between two layers of cellophane soaked in 2% glycerol solution (4.3.6.).

5.5.3. Staining of patanins

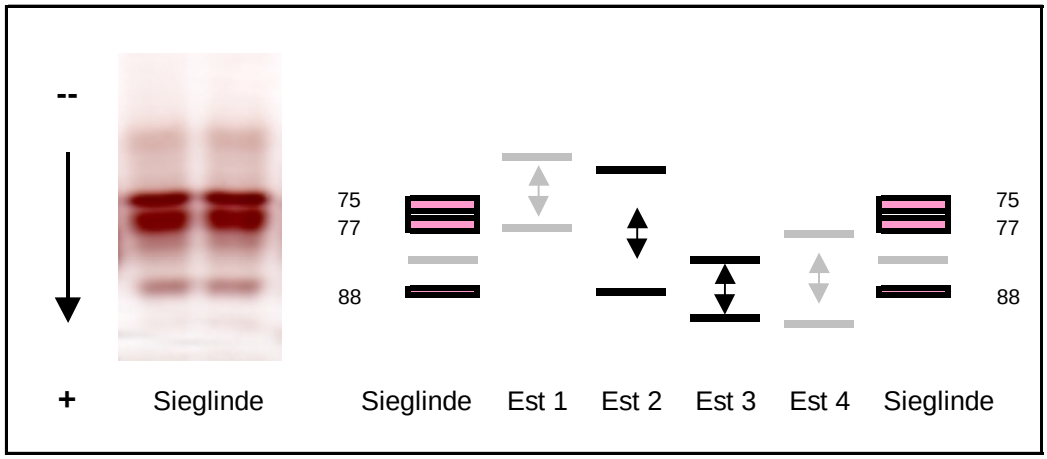
Gels from the PAGE pH 8.9 are marked, e.g. by cutting the gels corner. Then the gels are transferred in a staining container filled with 300 ml staining solution (4.3.2.) and incubated on a rocking platform shaker for 3 hours. The gels remain in the staining solution over night – without shaking. For destaining the gels are incubated on the shaker in normal water for 2 x 30 min. Finally the gels are incubated on the shaker in 2% glycerol solution (4.3.6.) for 30 min. After this incubation the gels are dried between two layers of cellophane soaked in 2% glycerol solution (4.3.6.).

6. Recognition of protein alleles

6.1. Recognition of the alleles encoding esterase isoenzymes

The positions of the individual esterase isoenzymes are calibrated by the variety Sieglinde. The variety Sieglinde shows three bands with high enzymatic activity in the following positions: 75 + 77 + 88.

The esterase isoenzymes of the potato tuber are extremely polymorphic. For a clear interpreation the zymogrammes are divided in four band blocks. The band blocks Est 1 and Est 4 have only a low enzymatic activity. The band blocks Est 2 and Est 3 have a strong enzymatic activity. Only Est 2 and Est 3 are used for assessment of distinctness, uniformity and stability.



Potatoes are vegetatively propagated tetraploid species. Therefore a lot of heterozygous genotypes can be expected. Individual genotypes can be distinct merely by the gene dosage. Such genotypes are often found in Est 2 and Est 3.

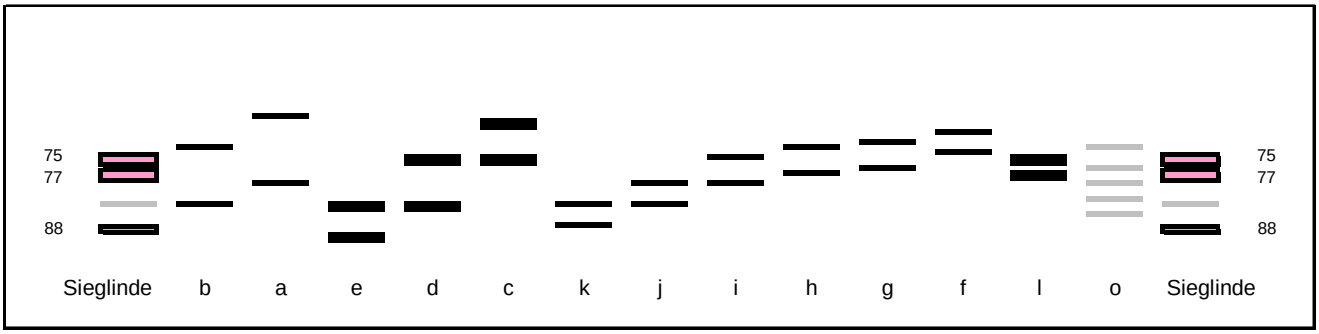
Combinations between null-allele and active alleles and genotpypes having the full gene dosage show identical bands. Therefore they are scored as identical.

Est 2	Est 3	Example variety	Note
a	o	Desiree	4
b	o	Cleopatra	18
c	o	Obelix	22
d	o	Achat	5
e	o	Sibu	20
f	o	Walli	12
h	o	Jetta	6
i	o	Renate	8
i	o	Hansa	1
k	o	Belita	13
l	o	Roxy	16
o	o	Ulla	11

Est 2	Est 3	Example variety	Note
d	b	Vital	23
g	b	Premiere	26
i	b	Selma	7
i	b	Ute	9

Est 2	Est 3	Example variety	Note
d	c	Krometa	19
i	c	Karakter	15
i	c	Karolin	3
k	d	Junior	17
l	c	Sieglinde	2

6.1.1. Schematization of the genotypes in Est 2

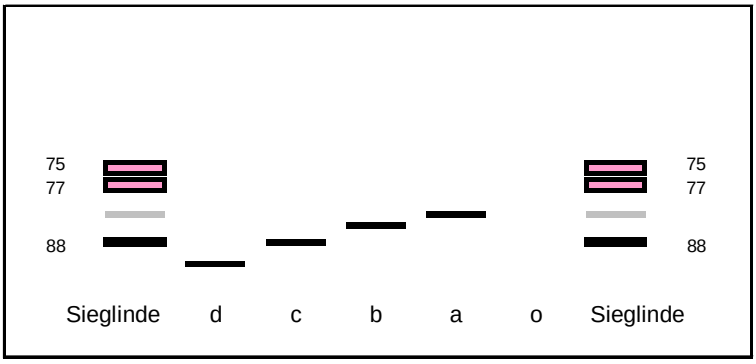


In Est 2 most genotypes show two bands (denomination: a –l). Sometimes genotypes with more than two bands are detectable. These types can be interpreted as combinations of two genotypes containing two bands.

Genotype in Est 2	Genotype in Est 3	Example variety	Remarks	Note
dl	o	Leyla	not distinguishable from genotype Est 2: d + Est 3: o	5
dl	c	Aiko	not distinguishable from genotype Est 2: d + Est 3: c	19
jf	o	Protea		27

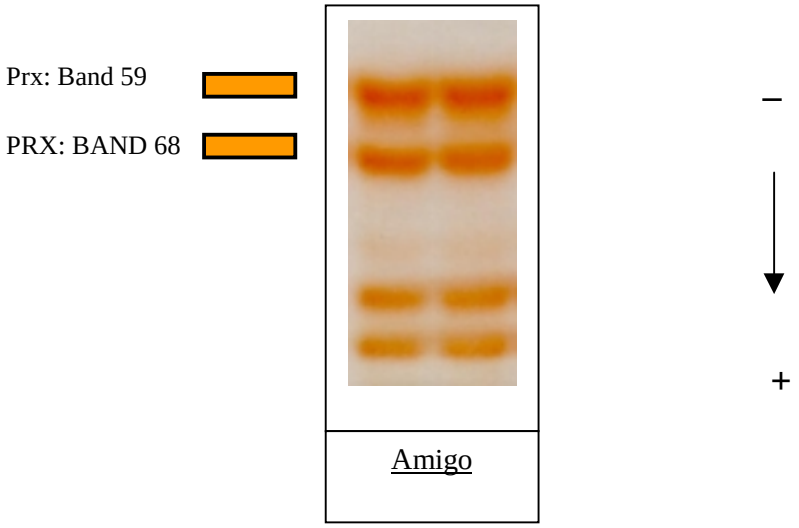
There is an overlapping of the gene products 75 and 77 encoded by genotype "Est 2: l" with gene product assigned to Est 1. Therefore it is not possible to have a clear separation between the bastard type "Est 2: l x d" and the genotype "Est 2: d". Therefore the genotype dl and the genotype l are not scored as different.

6.1.2. Schematization of the genotypes in Est 3



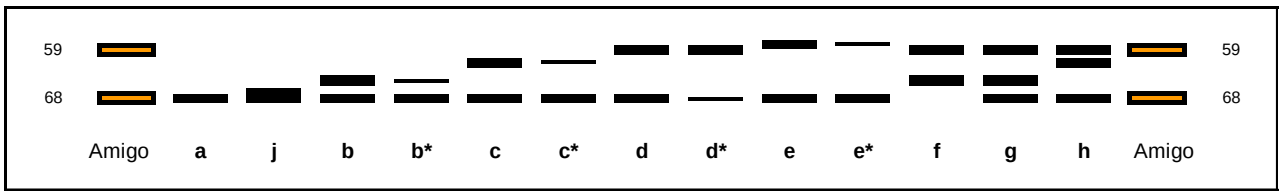
6.2. Recognition of the alleles encoding peroxydase isoenzymes

The peroxydase isoenzymes of the potato tuber are monomeric enzymes. The position of the individual peroxydase isoenzymes is calibrated by the variety Amigo. The variety Amigo shows two bands: 59 + 68.



Genotype	Example	Not
a	Hansa	1
b	Corine	2
c	Tomensa	3
d	Amigo	4
e	Jetta	5

Genotype	Example	Not
f	Diana	7
g	Thomana	6
h	Kanjer	8
j		1



Genotypes marked by an asterisk show decreased gene dosage in individual peroyxdases. They can be interpreted as combination between active alleles and the null-allele. Such genotypes are generally assigned to the genotypes with full gene dosage. The genotype j produces a zymogramme closely related to the genotype a; so the genotypes a and j are not scored as different. Both genotypes have the same note: note 1.

6.3. Recognition of the alleles encoding PAT

Patanins are monomeric peptide chains.

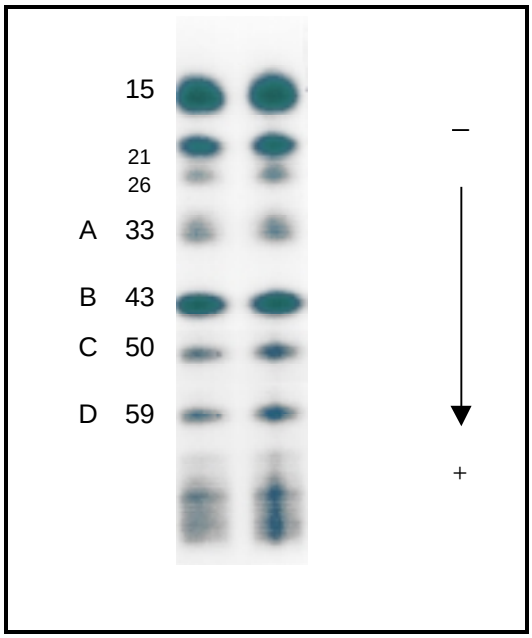
Genotype	Example variety	Note
1.01	**Secura**	**6**
2.01	**Erntestolz**	**9**
2.02	**Desiree**	**11**
2.03	**Pompadur**	**77**
2.04	**Delia**	**16**
3.01	**Quarta**	**19**
3.02	**Irmgard**	**21**
3.03	**Ulla**	**47**
3.04	**Fasan**	**49**
3.05	**Karolin**	**52**
3.06	**Gloria**	**61**
3.07	**Junior**	**58**
3.08	**Danva**	**26**
3.09	**Combi**	**50**
4.01	**Indira**	**31**
4.02	**Christa**	**34**
4.03	**Pia**	**54**
4.04	**Rubin**	**53**
4.05	**Cleopatra**	**65**
4.06	**Felsina**	**67**
4.07	**Cinja**	**43**

Genotype	Example variety	Note
4.08	**Sommergold**	**40**
4.09	**Walli**	**57**
4.10	**Juliver**	**27**
4.11	**Pepo**	**45**
4.12	**Saturna**	**42**
4.13		**84**
4.14	**Aiko**	**73**
4.15	**Amigo**	**75**
4.16		**85**
4.17	**Oktan**	**87**
5.01	**Belita**	**13**
5.02	**Solina**	**14**
6.01	**Artana**	**2**
6.02	**Quinta**	**7**
7.01	**Fausta**	**17**
7.02		**86**
7.03	**Pallina**	**25**
7.04	**Grata**	**20**
7.05	**Atica**	**23**
7.06	**Karnico**	**4**
7.07	**Franca**	**51**

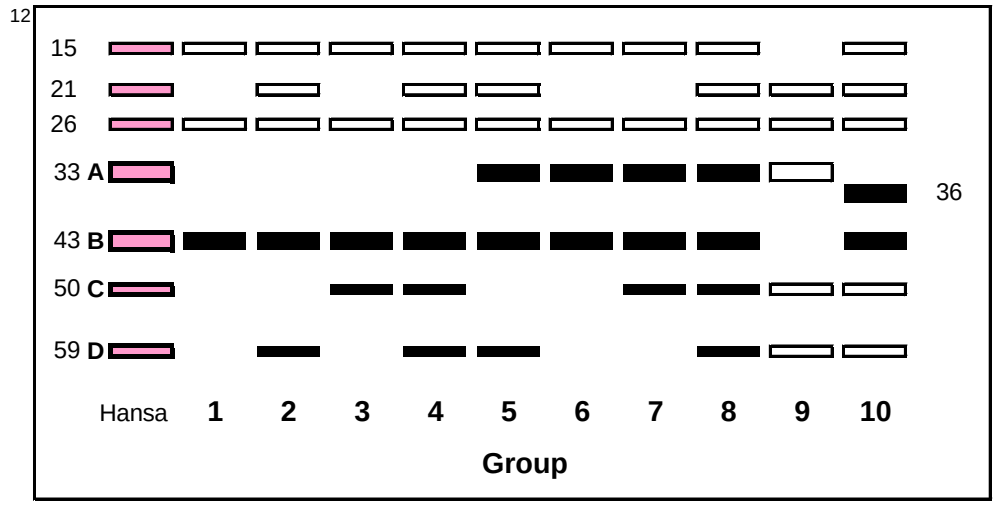
Genotype	Example variety	Note
7.08	**Adretta**	**60**
7.09	**Ukama**	**62**
8.01	**Berolina**	**18**
8.02	**Escort**	**35**
8.03	**Darwina**	**33**
8.04	**Shepody**	**55**
8.05	**Kardal**	**68**
8.06	**Padea**	**28**
8.07	**Elles**	**30**
8.08	**Solara**	**74**
8.09	**Thomana**	**76**
8.10	**Karida**	**29**
8.11	**Vebeca**	**44**
8.12	**Arnika**	**39**
8.13	**Krometa**	**37**
8.14	**Sirius**	**36**
8.15	**Alba**	**70**
8.16	**Feska**	**72**
9.01	**Calla**	**1**
10.01	**Liu**	**63**
10.02	**Kranich**	**80**

6.3.1. Grouping of the banding patterns

Patanins are defined by their electrophoretic mobility (REM-value). The positions of the individual patanins are illustrated by the example variety Hansa.

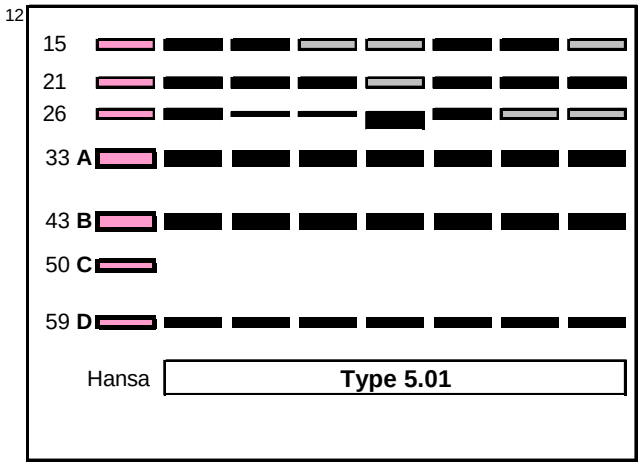


Patanins are extremely polymorphic proteins. The number of allele combinations is very high with more than 80. Therefore a grouping of the patanin patterns is neccessary. Patanins with high mobility (REM-value between 33 and 60) are used for grouping. These patanins are identical with the A-, B-, C- and D-bands by STEGEMANN and LÖSCHKE. They form 8 groups: group 1 – group 8. Additionally two special groups are existing: Group 10 is defined by the presence of the band 36 and group 9 is defined by the absence of the B-band.



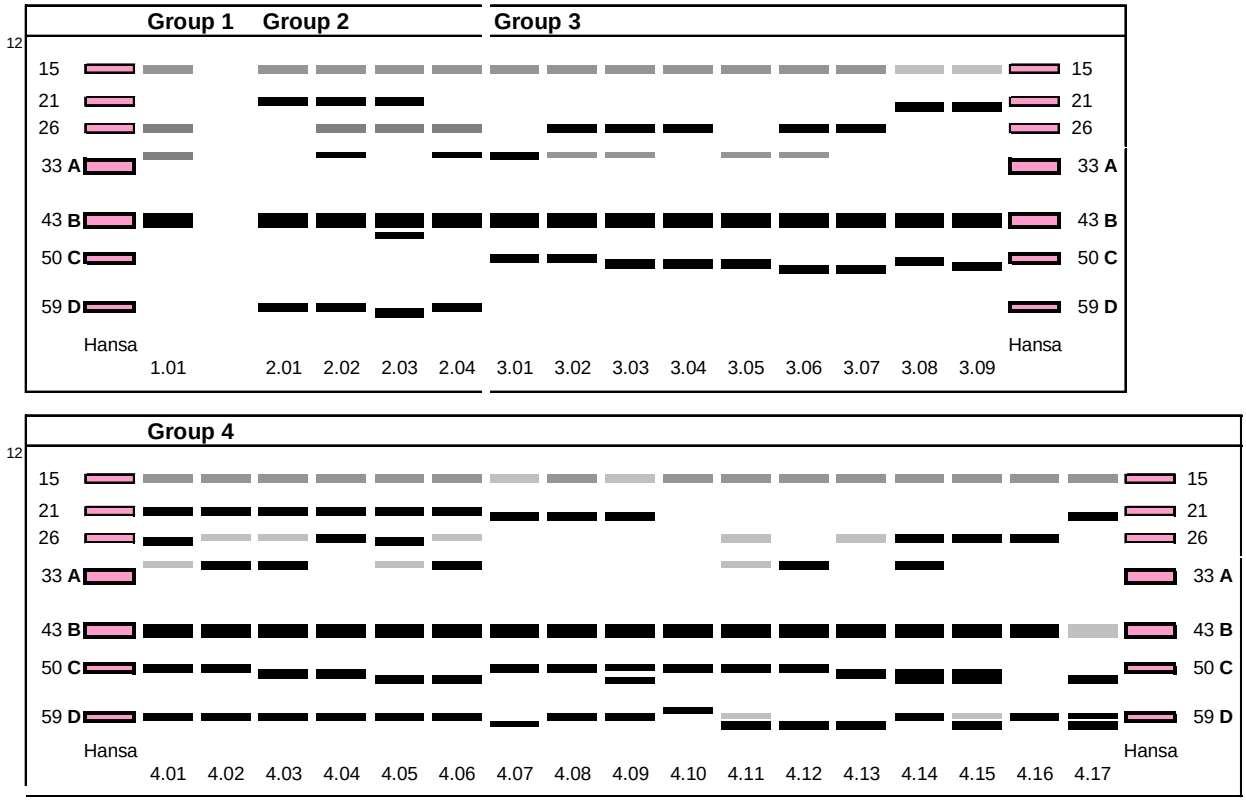
6.3.2. Analysis of the band intensity

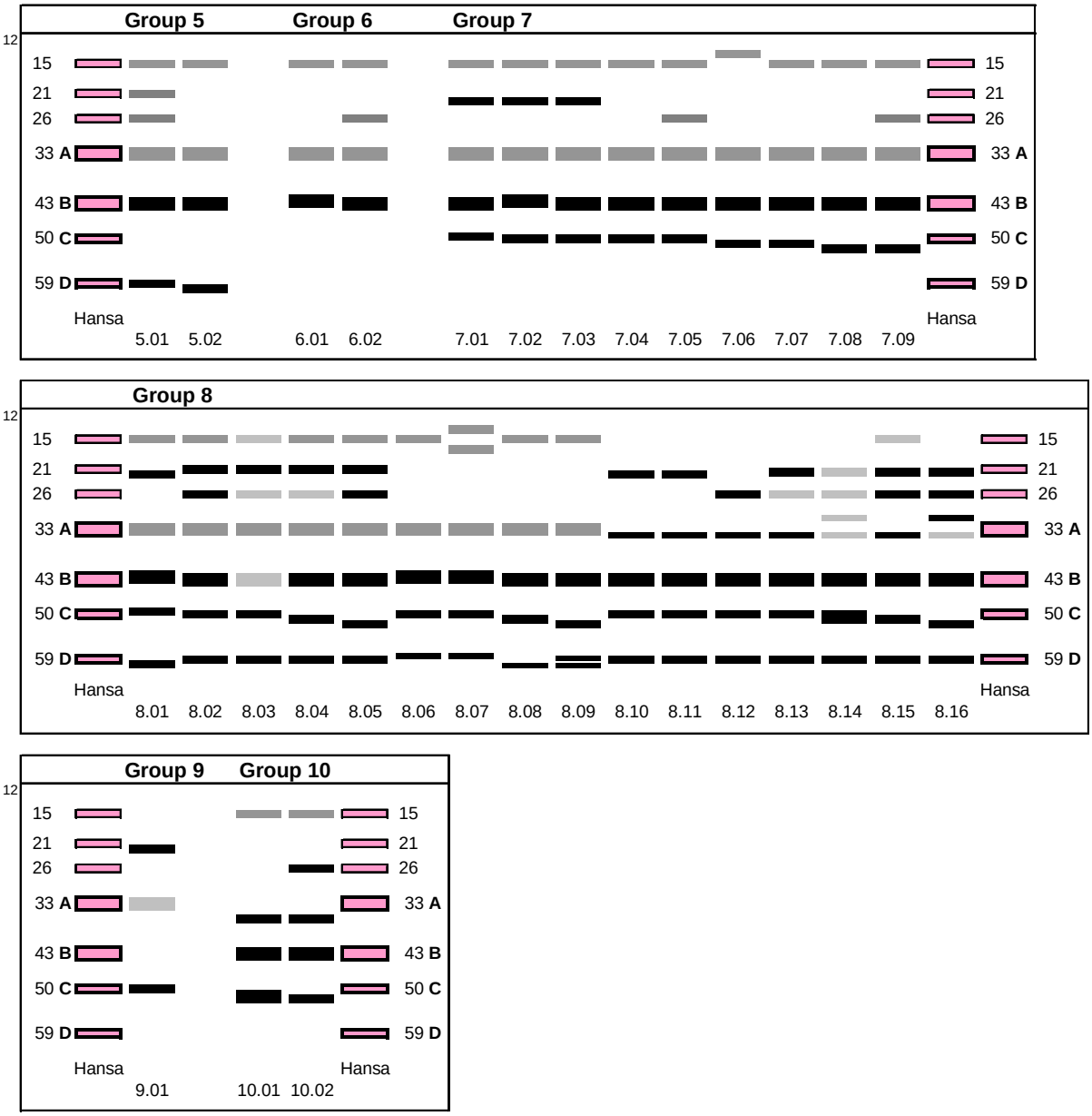
Differences in band intensities can be caused by different gene dosage. They are observed in the position 15, 21, 26, 31, 33 and 34. This occurs for example in type 5.01.



Patterns differing only in band intensities scored as identical.

6.3.3. Schematization of the banding patterns





Remarks to the groups 5 to 8

The band 33 is an extremely broad band and overlays the band 31; so in the presence of the band 33 the band 31 is not scorable. This is valid also in the case of a decreased band 33.

Literature

STEGEMANN; H. u. LOESCHKE, V.: Index of European Potato Varieties. Identification by electrophoretic Spectra, National registers, Appraisal of Characteristics, Genetic Data. Mitt. Biol. Bundesanst. Berlin-Dahlem, Heft 168, 1976.

[End of Annex and of document]