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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

POTATO

(*Solanum tuberosum* L.)

*

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

to be considered by the
Technical Working Party for Agricultural Crops at its thirty-second session,
to be held in Tsukuba, Japan, from September 8 to 12, 2003

Alternative Names:*

Latin	*English*	*French*	*German*	*Spanish*
**Solanum tuberosum* L.*, *S. tuberosum* L. *sensu lato*	Potato	Pomme de terre	Kartoffel	Papa, Patata

ASSOCIATED DOCUMENTS

These guidelines should be read in conjunction with document TG/1/3, "General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants" (hereinafter referred to as the "General Introduction") and its associated "TGP" documents.

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

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ANNEX 1

1. Subject of these Guidelines

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

2. Material Required

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

2.2 The material is to be supplied in the form of tubers, within the size range 35 to 50 mm.

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

100 tubers for each year of testing

2.4 The tubers supplied should be visibly healthy, not lacking in vigor or affected by any important pest or disease.

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

3. Method of Examination

3.1 Duration of Tests

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

3.2 Testing Place

The tests should normally be conducted at one place. If any characteristics of the variety, which are relevant for the examination of DUS, cannot be observed at that place, the variety may be tested at an additional place.

3.3 Conditions for Conducting the Examination

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

3.3.1 Timing of the examination

The optimum stage of development for the assessment of each characteristic is indicated by a number in the second column of the Table of Characteristics. The stages of development denoted by each number are described in Chapter 8.3.

3.3.2 Type of observation – visual or measurement

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

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

3.4 Test Design

3.4.1 Each test should be designed to result in a total of at least 60 plants, which should be divided between two or more replicates.

3.4.2 The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.5 Number of Plants / Parts of Plants to be Examined

Unless otherwise indicated, all observations should be made on 60 plants.

3.6 Additional Tests

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

4. Assessment of Distinctness, Uniformity and Stability

4.1 Distinctness

4.1.1 General Recommendations

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

4.1.2 Consistent Differences

The minimum duration of tests recommended in section 3.1 reflects, in general, the need to ensure that any differences in a characteristic are sufficiently consistent.

4.1.3 Clear Differences

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

4.2 Uniformity

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

4.2.2 For the assessment of uniformity, 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, 2 off-types are allowed. In the case of a sample size of 6 plants, 1 off-type is allowed.

4.3 Stability

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

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

5. Grouping of Varieties and Organization of the Growing Trial

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

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

5.3 The following have been agreed as useful grouping characteristics:

(a) Lightsprout: proportion of blue in anthocyanin coloration of base (characteristic 4)
(b) Flower corolla: intensity of anthocyanin coloration of inner side (characteristic 33)
(c) Flower corolla: proportion of blue in anthocyanin coloration of inner side (characteristic 34)
(d) Plant: time of maturity (characteristic 36)
(e) Tuber: color of skin (characteristic 39)

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

6. Introduction to the Table of Characteristics

6.1 Categories of Characteristics

6.1.1 Standard Test Guidelines Characteristics

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

6.1.2 Asterisked Characteristics

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

6.2 States of Expression and Corresponding Notes

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

6.3 Types of Expression

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

6.4 Example Varieties

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

6.5 Legend

(*) Asterisked characteristic – see Section 6.1.2

(QL) Qualitative characteristic – see Section 6.3
(QN) Quantitative characteristic – see Section 6.3
(PQ) Pseudo-Qualitative characteristic – see Section 6.3

(a) – (b) See Explanations on the Table of Characteristics in Chapter 8, Section 8.1
(+) See Explanations on the Table of Characteristics in Chapter 8, Section 8.2

Stage of development: see Section 3.3.1

VG-MG: see Section 3.3.2

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

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1	**VG (a)**	**Lightsprout: size**					
QN		small				Grata, Golden Wonder	3
		medium				Ute, Pentland Dell	5
		large				Gloria, Home Guard	7
2. (*) (+)	**VG (a)**	**Lightsprout: shape**					
PQ		spherical				Albas, Alpha	1
		ovoid				Gloria, Cara	2
		conical				Nicola, King Edward	3
		broad cylindrical				Cilena, Ulter Prince	4
		narrow cylindrical				Spunta	5
3. (*)	**VG (a)**	**Lightsprout: intensity of anthocyanin coloration of base**					
QN		absent or very weak				Estima	1
		weak				Karatop, Dunbar Standard	3
		medium				Grandifolia	5
		strong				Granola, Kerr's Pink	7
		very strong				Karida, Red Duke of York	9
4. (*) (+)	**VG (a)**	**Lightsprout: proportion of blue in anthocyanin coloration of base**					
QN		low				Cilena, Desiree	1
		medium				Pamina	2
		high				Agria, Record	3

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
5. **(*)**	**VG** **(a)**	**Lightsprout: pubescence of base**					
QN		absent or very weak				Croft	1
		weak				Hela, Pentland Dell	3
		medium				Alwara, Maris Piper	5
		strong				Rikea, Duke of York	7
		very strong				Dunluce	9
6.	**VG** **(a)**	**Lightsprout: size of tip in relation to base**					
QN		small				Quinta, Estina	3
		medium				Granola, King Edward	5
		large				Erntestolz, Dunbar Standard	7
7. **(+)**	**VG** **(a)**	**Lightsprout: habit of tip**					
QN		closed				Quinta, Estina	1
		intermediate				Rita, Catrona	3
		open				Premiere, Arran Pilot	5
8.	**VG** **(a)**	**Lightsprout: anthocyanin coloration of tip**					
QN		absent or very weak				Estima	1
		weak				Karatop, Duke of York	3
		medium				Planta, Spunta	5
		strong				Assia, Montana	7
		very strong				Red Duke of York	9

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
9.	**VG (a)**	**Lightsprout: pubescence of tip**					
QN		absent or very weak				Maris Piper	1
		weak				Cilena, Ulster Sceptre	3
		medium				Linda, Bintje	5
		strong				Agria, Vanessa	7
		very strong				Alcamaria	9
10. (*)	**VG (a)**	**Lightsprout: number of root tips**					
QN		few				Sanira, Craig's Royal	3
		medium				Nicola, Bintje, Cationa	5
		many				Belladonna, Maris Piper	7
11. (+)	**VG (a)**	**Lightsprout: length of lateral shoots**					
QN		short				Arkula, King Edward	3
		medium				Aiko, Kerr's Pink	5
		long				Quinta, Spunta	7
12. (+)	**1 VG**	**Plant: foliage structure**					
QN		stem type				Quarta, Kingston	1
		intermediate type				Desiree	2
		leaf type				Kennebec, Shannon	3
13. (*) (+)	**1 VG**	**Plant: growth habit**					
QN		upright				Quinta, Kerr's Pink	3
		semi-upright				Secura, Desiree	5
		spreading				Atica, Sante	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
14. (*) (+)	**1 VG**	**Stem: proportion of anthocyanin coloration**					
QN		absent or very small				Hela, Estima	1
		small				Marena, Atlantic	3
		medium				Saturna, Fianna	5
		large				Bimonda, Desiree	7
		very large				Redgem, Rooster	9
15. (+)	**1 VG**	**Leaf: outline size**					
QN		small				Natalie, Merlin, Kingston	3
		medium				Taiga, Claret	5
		large				Fausta, Kennebec	7
16. (+)	**1 VG**	**Leaf: openness**					
QN		closed				Likaria, Alisa, Red Pontiac	1
		intermediate				Ponto, Pentland Squire	3
		open				Grandifolia, Arran Consul	5
17. (+)	**1 VG**	**Leaf: presence of secondary leaflets**					
QN		weak				Solara, Wilja	3
		medium				Producent, Pentland Squire	5
		strong				Hercules, Cabaret, Cara	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
18. (+)	**1 VG**	**Leaf: green color**					
QN		light				Angela, Alisa, Yucon Gold	3
		medium				Ulme, Maris Piper, Merlin	5
		dark				Calla, Sierra, Navan	7
19. (+)	**1 VG**	**Leaf: proportion of anthocyanin coloration on midrib of upper side**					
QN		absent or very small				Grata, Cabaret, Estima	1
		small				Angela, Cara, Sierra	3
		medium				Camilla, Kerr's Pink	5
		large				Felicitas	7
		very large				Desiree, Roseval	9
20. (+)	**1 VG**	**Second pair of lateral leaflets: size**					
QN		very small				Inca Sun	1
		small				Kingston	3
		medium				Red Pontiac	5
		large				Shannon	7
		very large				Kennebec	9
21. (+)	**1 VG**	**Second pair of lateral leaflets: width in relation to length**					
QN		narrow				Othello, Yucon Gold	3
		medium				Desiree	5
		broad				Maxine, Wilja	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
22.	**1**	**Terminal and lateral leaflets: frequency of coalescence**					
(+)	**VG**						
QN		absent or very low				Cherie	1
		low				Palma, Valor	3
		medium				Freya, Isle of Jura	5
		high				Kolibri, Romano	7
		very high				Riviera	9
23.	**1**	**Leaflets: waviness of margin**					
(+)	**VG**						
QN		absent or very weak				Umatilla Russet	1
		weak				Grata, King Edward	3
		medium				Marabel, Verity	5
		strong				Aiko, Cabaret	7
		very strong				Friar, Waregem	9
24.	**1**	**Leaflets: depth of veins**					
(+)	**VG**						
QN		shallow				Pirol, Merlin	3
		medium				Premiere, Pomeroy	5
		deep				Santana, Bernadette	7
25.	**1**	**Leaflets: glossiness of the upperside**					
(+)	**VG**						
QN		dull				Satina, Maxine	3
		medium				Ute, Cabaret	5
		glossy				Christa, International Kidney, Nieta	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
26.	**1 VG**	**Leaflets: pubescence of blade at apical rosette**					
QL		absent				Zagadka	1
		present				Alena	9
27. (+)	**1 VG**	**Flower bud: proportion of anthocyanin coloration**					
QN		absent or very small				Grata, Estima	1
		small				Panda, Isle of Jura	3
		medium				Donella, Cara, Pomeroy	5
		large				Ponto, Valor	7
		very large				Argos, Inca Sun	9
28.	**2 VG**	**Plant: height**					
QN		very short				Swift, Mimi	1
		short				Atica, Saxon	3
		medium				Leyla	5
		tall				Grata, King Edward	7
		very tall				Tomba, Pomeroy	9
29. (*)	**2 VG**	**Plant: frequency of flowers**					
QN		absent or very low				Achat, King Edward	1
		low				Walli, Umatilla Russet, Estima	3
		medium				Rita	5
		high				Aiko, Maris Peer	7
		very high				Sibu, Pomeroy, Staney	9

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
30.	**2 VG**	**Inflorescence: size**					
(+)							
QN		small				Accent, Estima, Anna	3
		medium				Cilena, Nicola	5
		large				Karakter, Pomeroy	7
31.	**2 VG (b)**	**Inflorescence: proportion of anthocyanin coloration on peduncle**					
(+)							
QN		absent or very small				Lyra, Estima	1
		small				Liu, Pomeroy	3
		medium				Saturna, Maris Piper	5
		large				Desiree, Argos, Sarpo Mira	7
		very large				Stemster	9
32.	**2 VG**	**Flower corolla: size**					
(+)							
QN		very small				Rhona	1
		small				Sommergold, Kingston	3
		medium				Erntestolz, Verity	5
		large				Skala, Argos, Kerr's Pink	7
		very large				Rioja, Roseval	9

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
33. (*) (+)	**2 VG (b)**	**Flower corolla: intensity of anthocyanin coloration on inner side**					
QN		absent or very weak				Grata	1
		weak				Secura, Saxon	3
		medium				Ponto, Merlin	5
		strong				Producent, Pomeroy	7
		very strong				Argos	9
34. (*) (+)	**2 VG (b)**	**Flower corolla: proportion of blue in anthocyanin coloration on inner side**					
QN		low				Granola, Pomeroy, Rioja	1
		medium				Pamina, Cromwell, Sierra	2
		high				Karolin, Montose, Rocket	3
35. (*) (+)	**2 VG (b)**	**Flower corolla: proportion of coloration on inner side**					
QN		absent or very small				Kingston	1
		small				Laura	3
		medium				Berber, Barna	5
		large				Granola, Montrose	7
		very large				Ponto, Argos	9

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
36. **(*)** **(+)**	**3** **MG**	**Plant: time of maturity**					
QN		very early				Christa, Duke of York	1
		early				Cilena, Maris Peer	3
		medium				Nicola, Atlantic	5
		late				Aula, Maris Piper	7
		very late				Producent, Pomeroy	9
37. **(*)** **(+)**	**4** **VG**	**Tuber: shape**					
PQ		round				Mentor, Kerr's Pink, Rocket	1
		short-oval				Aula, Atlantic	2
		oval				Desiree	3
		long-oval				Linda, Pentland Dell	4
		long				Exquisa, Charlotte	5
		very long				Pompadour, Anja	6
38.	**4** **VG**	**Tuber: depth of eyes**					
QN		shallow				Fresco, Pentland Dell	3
		medium				Erntestolz, Kerr's Pink	5
		deep				Epicure	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
39. (*)	**4 VG**	**Tuber: color of skin**					
PQ		light beige				Nadine	1
		yellow				Cilena, Bernadette	2
		red				Desiree	3
		red parti-colored				King Edward	4
		blue				Heather	5
		blue parti-colored				Kestrel	6
		reddish brown				EV needed	7
40. (*)	**4 VG**	**Tuber: color of base of eye**					
PQ		white				Nadine	1
		yellow				Granola, Bernadette	2
		red				Quarta, Rioja	3
		blue				Heather	4
41. (*)	**4 VG**	**Tuber: color of flesh**					
PQ		white				Sibu, Rocket	1
		cream				Calla, Romano	2
		light yellow				Ukama,	3
		medium yellow				Quarta, Record	4
		dark yellow				Aula	5
		red				Red Salad	6
		red parti-colored					7
		blue				Congo	8
		blue parti-colored					9

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
42. **(+)**	**4** **VG**	**<u>Light beige and yellow skinned varieties only:</u>** **Tuber: anthocyanin coloration of skin in reaction to light**					
QN		absent or very weak				Agata, Estima	1
		weak				Fausta, Pentland Ivory	3
		medium				Linda, Pentland Dell	5
		strong				Palma, Epicure	7
		very strong					9

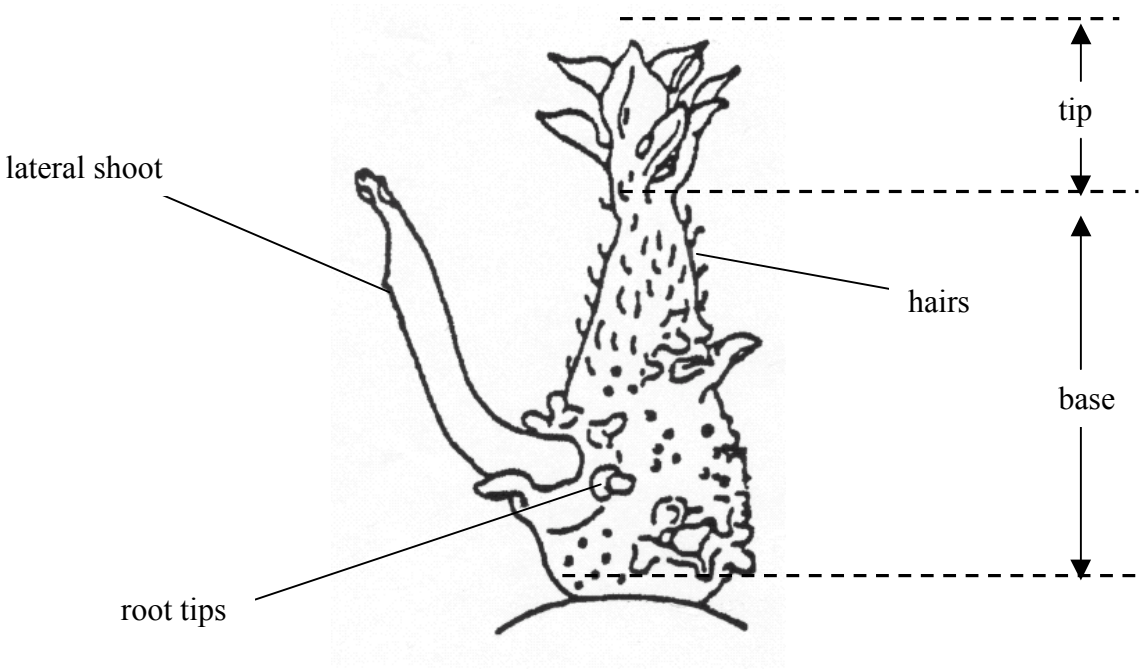
8. Explanations on the Table of Characteristics

8.1 Explanations covering several characteristics

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

(a) Lightsprout: All observations on the lightsprout should be made on a total of 6 tubers as a minimum according to the following method:

Lightsprout

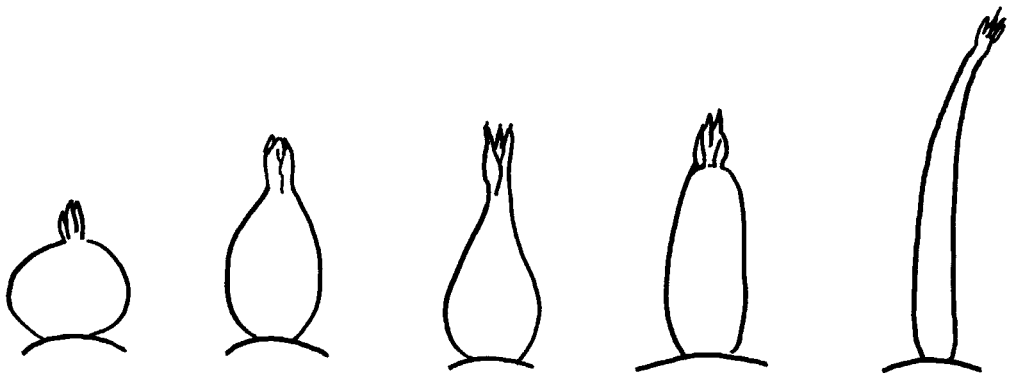


The spectrum and the intensity of the light source are the most determining factors for the expression of characteristics of lightsprouts. This spectrum is unambiguously defined by the type of lamps and the voltage used. When extremes are avoided the influence of the temperature on the speed of development is small. A good expression of characteristics is obtained with lightsprouts growing in a cabinet at room temperature under exclusion of day light and under continuous light of small incandescent bulbs (6V AC / 0.05 A, 8 per square meter, 25-40 cm above the tubers).

(b) Flower: All observations of flower color should be made on freshly opened flowers

8.2 Explanations for individual characteristics

Ad. 2: Lightsprout: shape

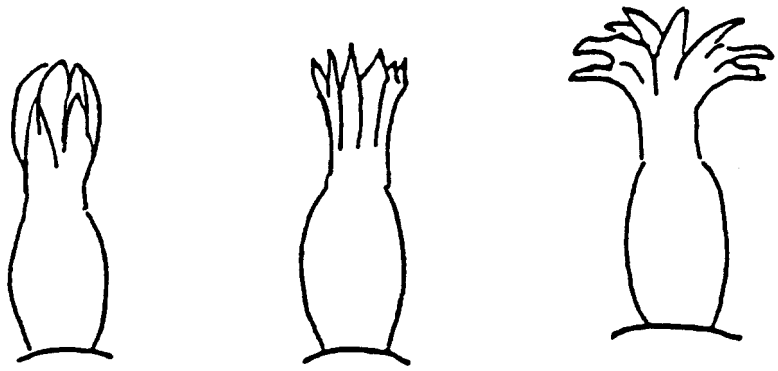


1	2	3	4	5
spherical	ovoid	conical	broad cylindrical	narrow cylindrical

Ad. 4: Lightsprout: proportions of blue in anthocyanin coloration of base,
and 34: Flower corolla: proportion of blue in anthocyanin coloration on inner side

The color of anthocyanin results from a red and a blue component. If the proportion of blue is low the anthocyanin appears red-violet. If the proportion of blue is high the anthocyanin appears blue-violet.

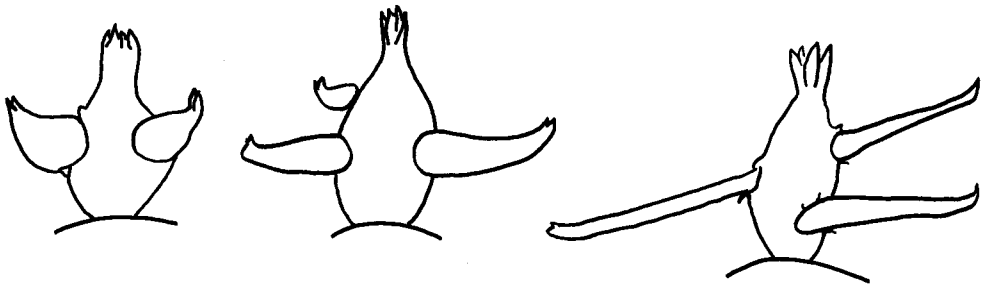
Ad. 7: Lightsprout: habit of tip



1	3	5
closed	intermediate	open

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

Ad. 11: Lightsprout: length of lateral shoots



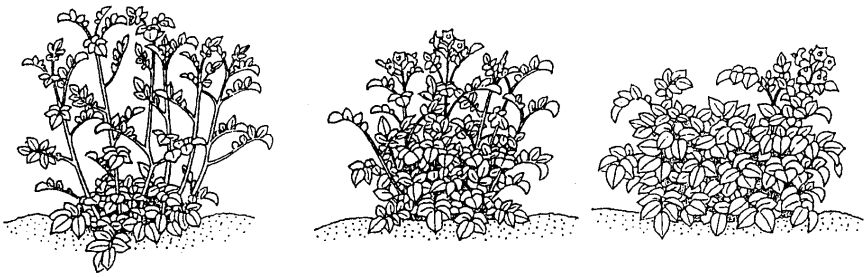
3	5	7
short	medium	long

Ad. 12: Plant: foliage structure

Stem type: foliage open, stems clearly visible

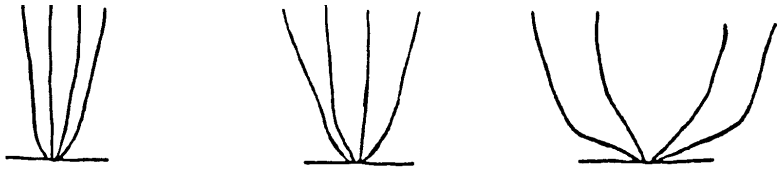
Intermediate type: foliage half open, stems partly visible

Leaf type: foliage closed, stems not, or hardly, visible



1	2	3
stem type	intermediate type	leaf type

Ad. 13: Plant: growth habit



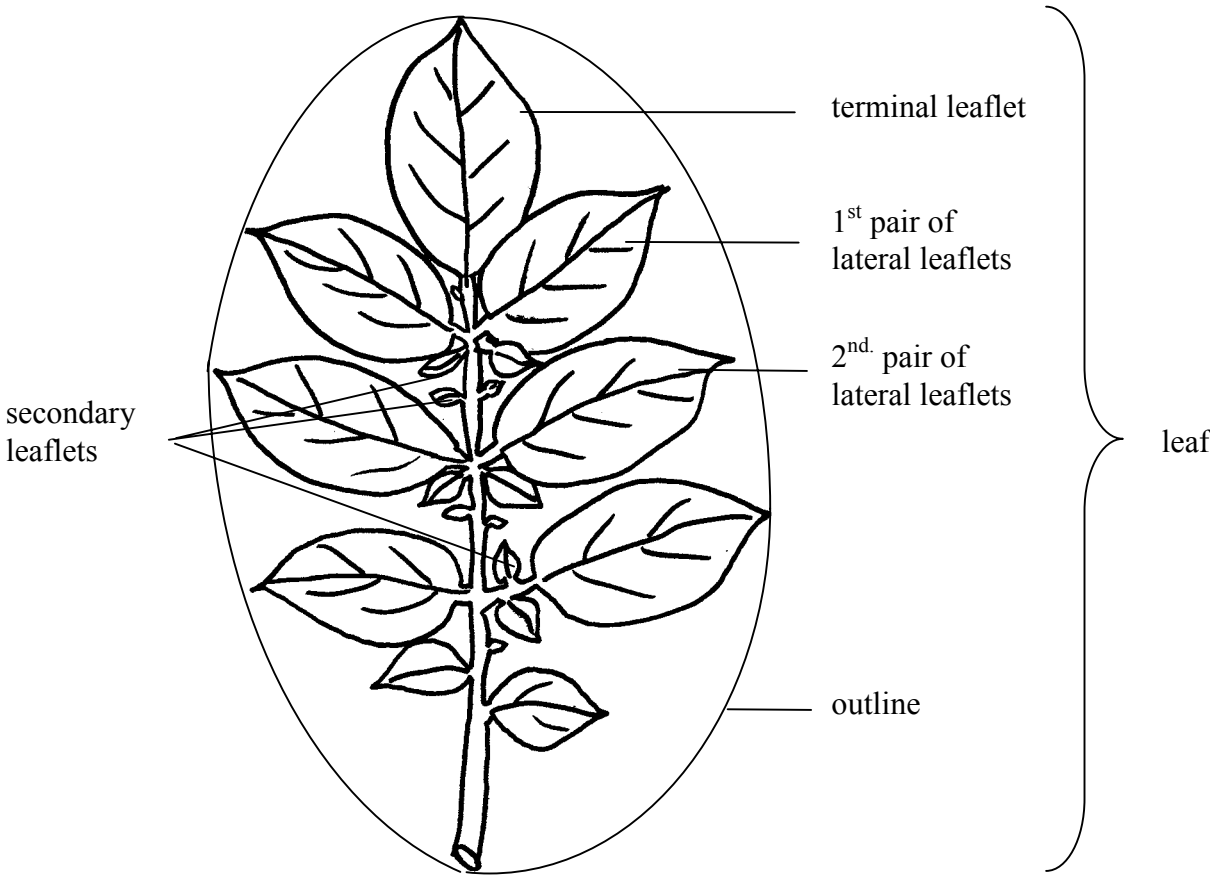
3	5	7
upright	semi-upright	spreading

Ads. 14, 19, 27, 31, 34: Proportion of anthocyanin coloration

The proportion of anthocyanin coloration should be observed in relation to the total area. Distribution and intensity should not be considered.

The proportion of anthocyanin coloration of flower buds should be observed on fully developed buds before the corolla is visible.

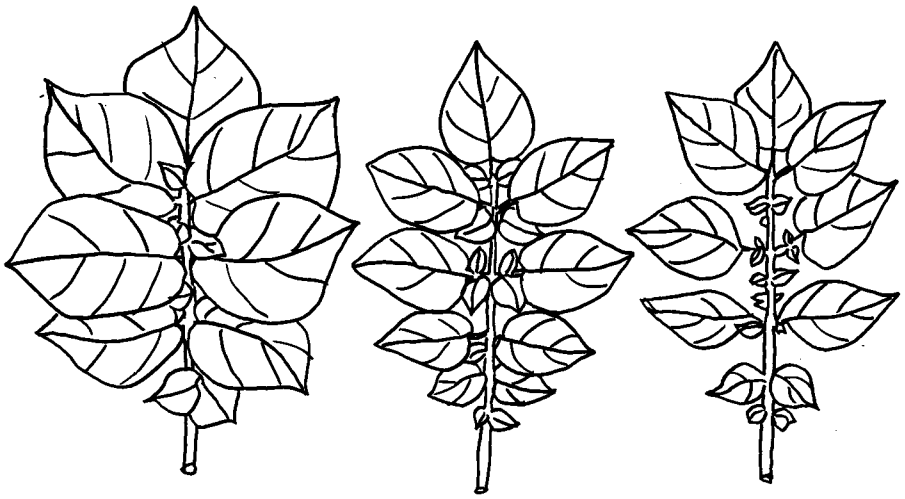
Ads. 15 to 25: Leaf characteristics



All observations on the leaf should be made on fully developed leaves from the centre of the plant.

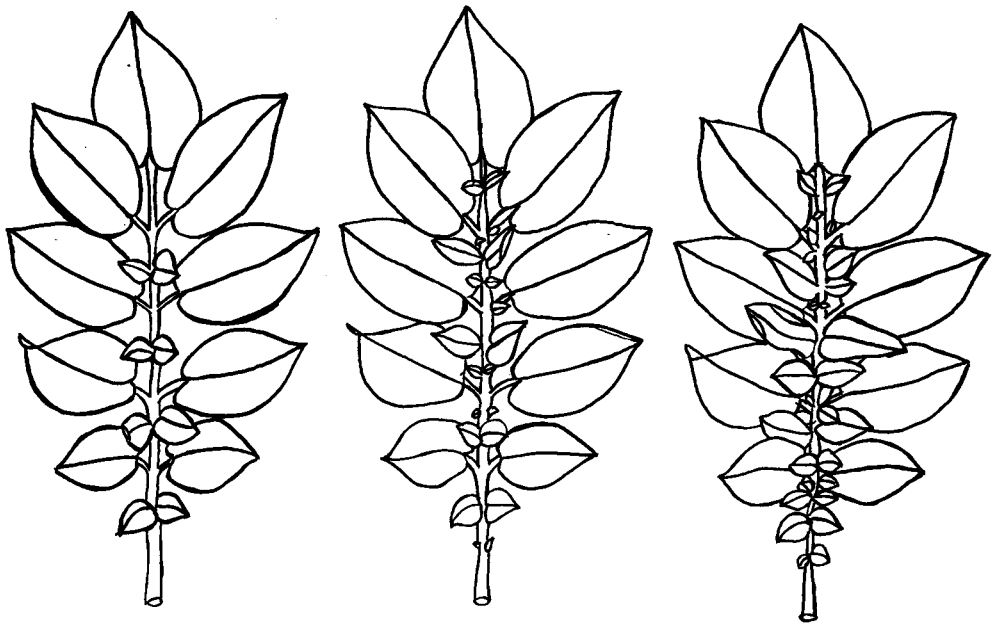
For the observation of characteristic 15, 16, 17 and 20 one leaf from each of 20 plants should be picked from a main stem midway between the top and the bottom of the plant.

Ad. 16: Leaf: openness



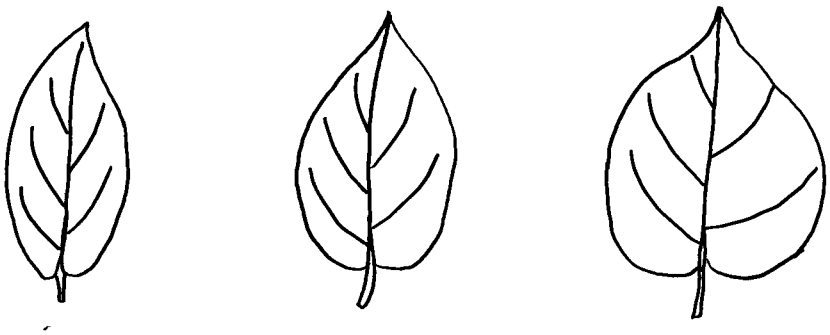
1	3	5
closed	intermediate	open

Ad. 17: Leaf: presence of secondary leaflets



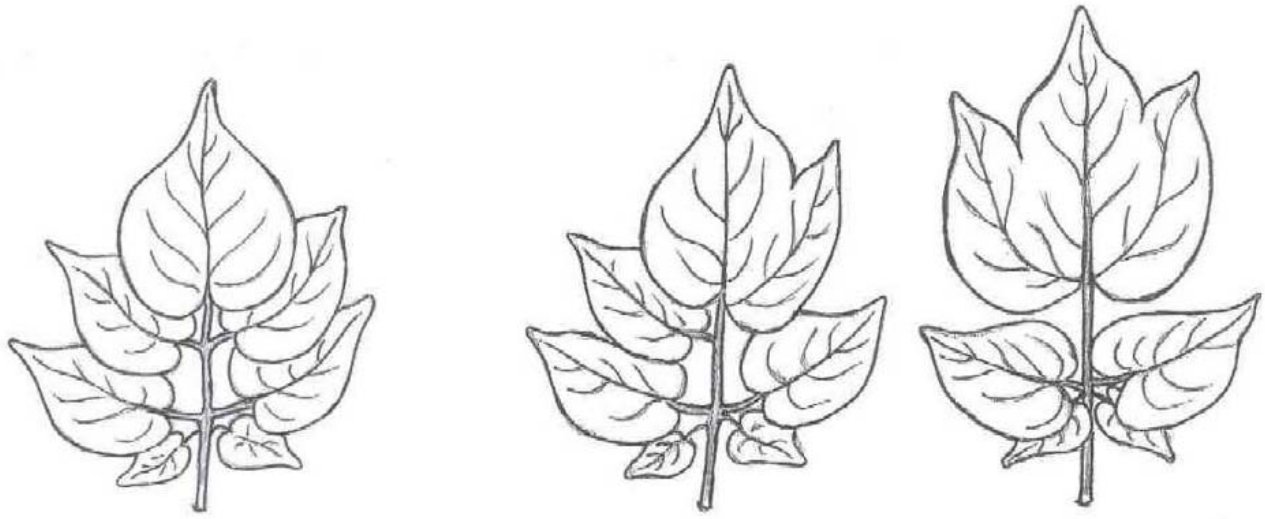
3	5	7
weak	medium	strong

Ad. 21: Second pair of lateral leaflets: width in relation to length



3	5	7
narrow	medium	broad

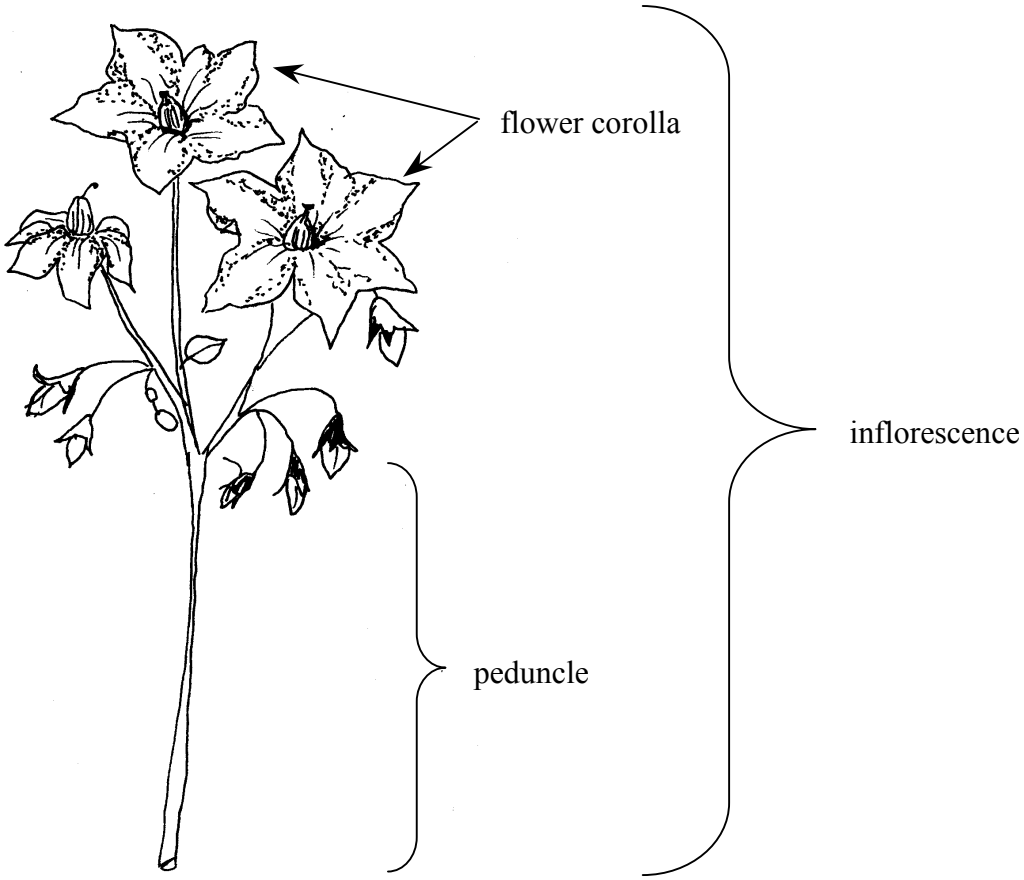
Ad. 22: Terminal and lateral leaflets: frequency of coalescence



not coalescent coalescent

The proportion of coalescent leaves should be observed.

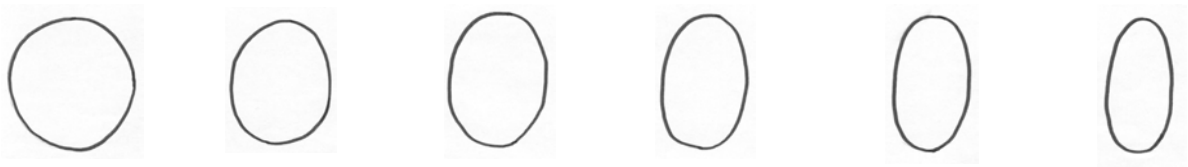
Ads. 30–35: Flower characteristics



Ad. 36: Plant: time of maturity

The time of maturity is reached when 80% of the leaves are dead.

Ad. 37: Tuber: shape



1	2	3	4	5	6
round	short-oval	oval	long-oval	long	very long

The average shape should be observed on the harvested material from each plot.

Ad. 42: Light beige and yellow skinned varieties only: Tuber: anthocyanin coloration of skin in reaction to light

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

8.3 Optimal Stage of Assessment of Characteristics

1 = bud stage
2 = flowering stage
3 = ripening stage of tubers
4 = after harvest

9. Literature

10. Technical Questionnaire

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

	Application date: (not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE
to be completed in connection with an application for plant breeders' rights

1. Subject of the Technical Questionnaire

1.1 *Latin Name*	*Solanum tuberosum* L.
1.2 Common Name	Potato

2. Applicant

Name	
Address	
Telephone No.	
Fax No.	
E-mail address	

Breeder (if different from applicant)

3. Proposed denomination and breeder's reference

Proposed denomination (if available)	
Breeder's reference	

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

4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]
(please state parent varieties)
(b) partially known cross [ ]
(please state known parent variety(ies))
(c) totally unknown cross [ ]

4.1.2 Mutation [ ]
(please state parent variety)

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

4.1.4 Other [ ]
(please provide details)

4.2 Method of propagating the variety

4.2.1 Vegetative propagation

(a) tubers [ ]

(b) other (state method) [ ]

4.2.2 Seed [ ]

4.2.3 Other [ ]
(please provide details)

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

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

	Characteristics	Example Varieties	Note
5.1 (4)	**Lightsprout: proportion of blue in anthocyanin coloration of base**		
	low	Cilena, Desiree	1[ ]
	medium	Pamina	2[ ]
	high	Agria, Record	3[ ]
5.2 (29)	**Plant: frequency of flowers**		
	absent or very low	Achat, King Edward	1[ ]
	low	Walli, Umatilla Russet, Estima	3[ ]
	medium	Rita	5[ ]
	high	Aiko, Maris Peer	7[ ]
	very high	Sibu, Pomeroy, Staney	9[ ]
5.3 (33)	**Flower corolla: intensity of anthocyanin coloration on inner side**		
	absent or very weak	Grata	1[ ]
	weak	Secura, Saxon	3[ ]
	medium	Ponto, Merlin	5[ ]
	strong	Producent, Pomeroy	7[ ]
	very strong	Argos	9[ ]
5.4 (34)	**Flower corolla: proportion of blue in anthocyanin coloration on inner side**		
	low	Granola, Pomeroy, Rioja	1[ ]
	medium	Pamina, Cromwell, Sierra	2[ ]
	high	Karolin, Montose, Rocket	3[ ]

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

	Characteristics	Example Varieties	Note
5.5 (36)	**Plant: time of maturity**		
	very early	Christa, Duke of York	1[ ]
	early	Cilena, Maris Peer	3[ ]
	medium	Nicola, Atlantic	5[ ]
	late	Aula, Maris Piper	7[ ]
	very late	Producent, Pomeroy	9[ ]
5.6 (37)	**Tuber: shape**		
	round	Mentor, Kerr's Pink, Rocket	1[ ]
	short-oval	Aula, Atlantic	2[ ]
	oval	Desiree	3[ ]
	long-oval	Linda, Pentland Dell	4[ ]
	long	Exquisa, Charlotte	5[ ]
	very long	Pompadour, Anja	6[ ]
5.7 (39)	**Tuber: color of skin**		
	light beige	Nadine	1[ ]
	yellow	Cilena, Bernadette	2[ ]
	red	Desiree	3[ ]
	red parti-colored	King Edward	4[ ]
	blue	Heather	5[ ]
	blue parti-colored	Kestrel	6[ ]
	reddish blue	EV needed	7[ ]

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

	Characteristics	Example Varieties	Note
5.8 (40)	**Tuber: color of base of eye**		
	white	Nadine	1[ ]
	yellow	Granola, Bernadette	2[ ]
	red	Quarta, Rioja	3[ ]
	blue	Heather	4[ ]
5.10 (41)	**Tuber: color of flesh**		
	white	Sibu, Rocket	1[ ]
	cream	Calla, Romano	2[ ]
	light yellow	Ukama,	3[ ]
	medium yellow	Quarta, Record	4[ ]
	dark yellow	Aula	5[ ]
	red	Red Salad	6[ ]
	red parti-colored		7[ ]
	blue	Congo	8[ ]
	blue parti-colored		9[ ]

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

6. Similar varieties and differences from these varieties

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

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the **similar** variety(ies)	Describe the expression of the characteristic(s) for **your** candidate variety
Example		*(example to be inserted)*	*(example to be inserted)*

Comments:

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

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

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

Yes [ ] No [ ]

(If yes, please provide details)

7.2 Special conditions for the examination of the variety

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

Yes [ ] No [ ]

7.2.2 If yes, please give details:

7.3 Other information

8. Authorization for release

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

Yes [ ] No [ ]

(b) Has such authorization been obtained?

Yes [ ] No [ ]

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

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

9. Information on plant material to be examined.

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

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

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

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

(c) Tissue culture Yes [ ] No [ ]

(d) Other factors Yes [ ] No [ ]

Please provide details of where you have indicated "yes".

……

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

Applicant's name

Signature Date

[Annex follows]

ANNEX

ADDITIONAL USEFUL EXPLANATIONS

Table of Contents | Page

Part I	Introduction	2
Part II	Characteristics Derived by Using Electrophoresis	3
Part III	Description of the Method to be Used	6

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.

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
43.	**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

	Stage [1] / Stade [1] / Stadium [1] / Estado [1] English	français	deutsch	español	Example Varieties / Exemples / Beispielssorten / Variedades ejemplo	Note/ Nota
44.	**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
45.	**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 2.02				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
45. (cont.)	**Allele expression at locus Pat (cont.)**					
	Genotype 8.06				Padea	28
	Genotype 8.10				Kanjer	29
	Genotype 8.07				Elles	30
	Genotype 4.01				Indira	31
	Genotype 8.02				Hansa	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 4.03				Pia	54
	Genotype 8.04				Shepody	55
	Genotype 4.09				Walli	57
	Genotype 3.07				Junior	58

	Stage [1] / Stade [1] / Stadium [1] / Estado [1] English	français	deutsch	español	Example Varieties / Exemples / Beispielssorten / Variedades ejemplo	Note/ Nota
45. (cont.)	**Allele expression at locus Pat (cont.)**					
	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 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 isoenzymes and storage proteins in potatoes

1. Number of tubers per test

- for distinctness, uniformity and stability: 6 tubers

The tubers should be mature, preferably harvested after senescence of foliage. Tubers stored between 4 -10° C can be used for the analysis of peroxidases and patatins as long as there is no, or only slight, sprouting. The activity of esterases can decrease during storage of tubers. Therefore, fresh tubers should be used for the analysis of esterases.

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 **(Safety advice**: Acrylamide is an extremely toxic chemical! For safety reasons a commercial solution should be used.)
Ammonium persulfate (APS)
2% Bisacrylamide (**Safety advice**: For safety reasons a commercial solution should be used.)
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 **(Safety 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	Quantity	Remark
4.1.1	Extraction solution A	Sodium sulphite Sodium disulphite de-ionised water	5.00 g 3.75 g ad 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	Quantity	Remark
4.2.1.1	Gel buffer stock solution	TRIS Boric acid de-ionised water	30.26 g 36.60 g ad 1000 ml	
4.2.1.2	2% APS solution	Ammonium persulfate de-ionised water	1 g ad 50 ml	to be prepared daily
4.2.1.3	Tank buffer	Gel buffer stock solution (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 peroxidases and patatins

No.	*Solution*	Ingredients	Quantity	Remark
4.2.2.1	Resolving gel buffer	TRIS de-ionised water	75.4 g ad 1000 ml	to be adjusted at 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	to be adjusted at pH 6.7 with HCl
4.2.2.3	Stacking gel preparing solution	Stacking gel buffer (4.2.2.2.) 40% Acrylamide 2% Bisacrylamide de-ionised water Sucrose	280 ml 45 ml 73 ml 150 ml 80 g	
4.2.2.4	2% APS solution	Ammonium persulfate de-ionised water	0.4 g 20 ml	to be prepared daily
4.2.2.5	10 % Ethanol	Ethanol de-ionised water	10 ml ad 100 ml	
4.2.2.6	Tank buffer stock solution	TRIS Glycine de-ionised water	103 g 70 g ad 1000 ml	
4.2.2.7	Tank buffer	Tank buffer stock solution (4.2.2.6.) de-ionised water	50 ml ad 1000 ml	to be prepared daily

4.3. Staining Solutions for patatins, peroxidases and esterases

No.	*Solution*	Ingredients	Quantity	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 hour and to be shaken before use
4.3.2	Staining solution for patatins	TCA Glacial acetic acid Water Methanol Stock solution (4.3.1.)	240 g 280 ml 3300 ml 600 ml 100 ml	
4.3.3	Staining buffer A	Na_2HPO_4 x $12H_2O$ de-ionised water	53.7 g ad 1000 ml	
4.3.4	Staining buffer B	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 % Glycerol	Glycerol Water	20 g ad 1000 ml	

5. Procedure

5.1 Preparation of the sample

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

The thawed tubers are cut in two halves and squeezed by hand. 1.5 ml of the sap are mixed with 0.4 ml extraction solution C (4.1.3) in 2 ml microtubes. Next the solution is centrifuged for 15 min at 3000 rpm at 10° C. The clear supernatants are transferred into new microtubes and can be stored at – 18 °C.

5.2 Preparation of the gels

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

Preparation of about 120 ml gel solution (T: 4.9%; C: 4.7%):

Under stirring 50 mg sodium sulphite are dissolved in 76 ml de-ionised water. The following solutions are added:

14 ml Gel buffer stock solution (4.2.1.1),
14 ml 40% Acrylamide
14 ml 2% BIS.

Polymerisation is incluced by addition of
0.6 ml DMAPN and
2.0 ml 2% APS solution (4.2.1.2).

The gel cassettes are assembled and the gels are poured according to the users manual of the equipment, avoiding the formation of air bubbles. Polymerisation should take place at room temperature for at least 15 min. After removal of the mould-forming combs the moulds are rinsed with tank buffer (4.2.1.3).

5.2.2. Preparation of the gels for PAGE pH 8.9 of the peroxidases and patatins

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 slow stirring:

60 ml resolving gel buffer (4.2.2.1),
14 ml de-ionised water,
14 ml 40% Acrylamide
13 ml 2% Bisacrylamide

Polymerisation is included by addition of
100 µl TEMED and
6 ml 2% APS solution (4.2.2.4)

The gel cassettes are assembled and the gels are poured according to the users manual of the equipment, avoiding the formation of air bubbles. Polymerisation should take place at room temperature for at least 15 min. The gel cassettes should not be filled entirely, in order to leave space for a 14 mm layer of stacking gel. The gel is overlapped with 10% ethanol (4.2.2.5) using a syringe. When the polymerisation is finished, the gel surface is rinsed in the cassette 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 (4.2.2.4) are mixed under slow stirring.

The gels are poured according to the users manual of the equipment, avoiding the formation of air bubbles. Polymerisation should take place at room temperature for about 15 min. After removal of the mould-forming combs the moulds are rinsed with tank buffer (4.2.2.7).

5.3 Electrophoresis

5.3.1 Sample loading

For esterases and peroxidases each slot should be filled with 6 µl - 12 µl extract depending on the slot size. For patatins 3 µl - 6 µl extract should be used per slot depending on the slot size.

5.3.2 Conditions for PAGE pH 7.9 of the esterases

Tank buffer	= Solution 4.2.1.3
Current (gel 11 cm broad, 1 mm thick)	= first 10 min 10 mA, then 20 mA
Voltage	= max. 300 V
Temperature	= 5° C to 15° C
Migration way	= from cathode (-) to anode (+)
Migration distance	= 6 cm Amido black

5.3.3 Conditions for PAGE pH 8.9 of the peroxidases and patatins

Tank buffer	= Solution 4.2.2.7.
Current (gel 11 cm broad, 1 mm thick)	= first 10 min 10 mA, then 20 mA
Voltage	= max. 300 V
Temperature	= 5° C to 15° C
Migration way	= from cathode (-) to anode (+)
Migration distance	= 6 cm Bromphenol blue

5.4 Staining

5.4.1. Staining of esterases

Gels from the PAGE pH 8.9 are marked, e.g. by cutting the gels corner. 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 are incubated on a rocking platform shaker. 50 mg 1-Naphthylacetate are dissolved in 3 drops acetone and diluted with de-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 de-ionised 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 de-ionised water for 2 x 30 min. Finally the gels are incubated on the shaker in 2% glycerol (4.3.6) for 30 min. After this incubation the gels are dried between cellophane sheets soaked in 2% glycerol.

5.4.2. Staining of peroxidases

Gels from the PAGE pH 8.9 are marked, e.g. by cutting the gels corner. The gels are transferred in a staining container filled with 200 ml staining buffer B (4.3.4) and are 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 de-ionised water for 2 x 30 min. Finally the gels are incubated on the shaker in 2% glycerol (4.3.6) for 30 min. After this incubation the gels are dried between cellophane sheets soaked in 2% glycerol.

5.4.3. Staining of patatins

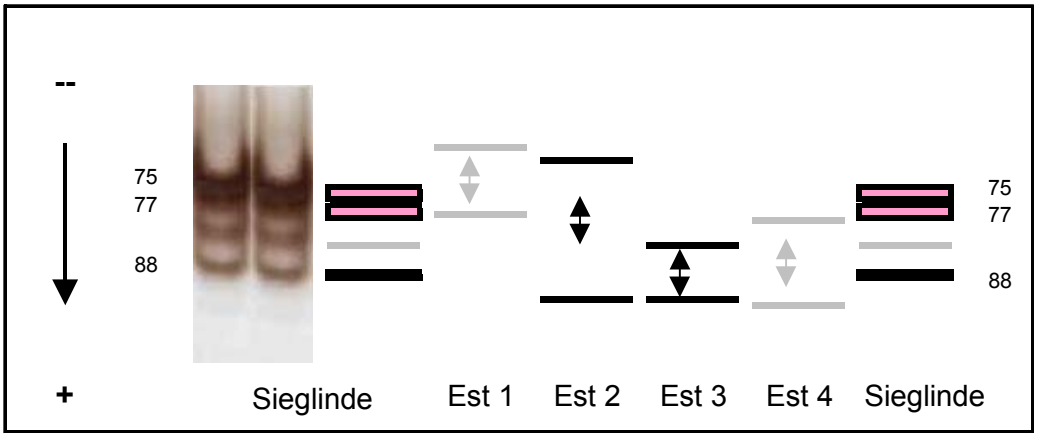
Gels from the PAGE pH 8.9 are marked, e.g. by cutting the gels corner. The gels are transferred in a staining container filled with 300 ml staining solution (4.3.2) and incubated on a rocking platform shaker for 3 hours. The gels remain in the staining solution over night without shaking. For destaining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

6. Recognition of protein alleles

6.1. Recognition of the alleles encoding esterase isoenzymes

Esterase of the potato tuber is a hightly polymorphic monomoric enzyme. For a clear interpretation the zymogrammes are divided in four band blocks according to the combined occurence of bands. 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 should be used for the assessment of distinctness, uniformity and stability.

The position of the individual esterase isoenzymes is calibrated according the variety Sieglinde. The variety Sieglinde has three bands with high enzymatic activity in the positions 75, 77 and 88.



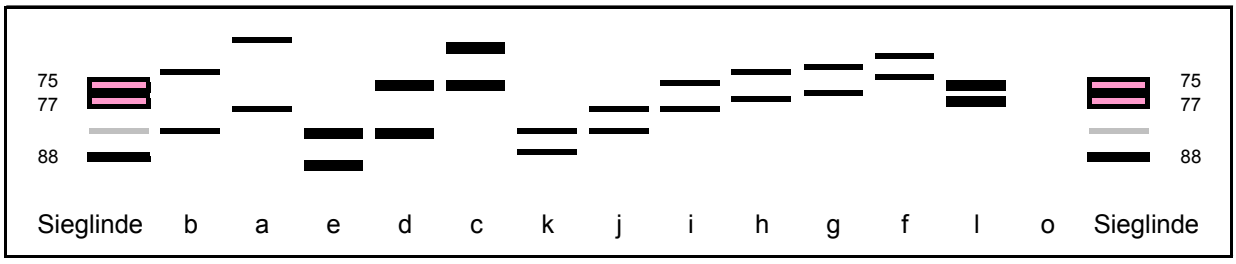
Most genotypes express two bands in Est 2 and one band in Est 3. For both regions there are also genotypes without bands which are considered as null alleles. A few genotypes have more than two bands in Est 2. They can be described as a combination of two genotypes with two bands. In some varieties the expression "dl" can be observed in Est 2. Because of an overlap of band Est 2-77 with a weak band Est 1-77 the type "dl" can not clearly be separated from "d" and it is not considered as a distinct type.

Differences in the intensity of bands assigned to Est 2 and Est 3 are not considered as clear differences. Corresponding genotypes are scored with the same note.

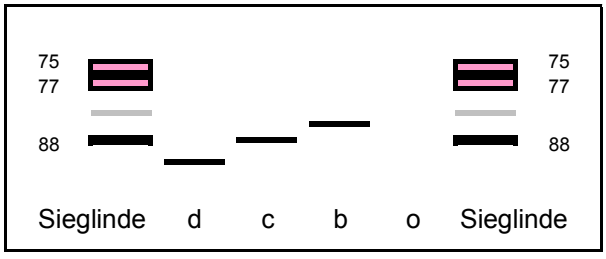
A List of all described genotypes, example varieties and corresponding notes is proveded in the following table. For schematization of band patterns see 6.1.1 and 6.1.2.

Genotype *Est 2* + *Est 3*			***Band Position*** *Est 2* + *Est 3*			**Example Variety**	***Note***
a	+	o	66+79			Desiree	4
b	+	o	72+83			Cleopatra	18
c	+	o	68+75			Obelix	22
d	+	o	75+83			Achat	5
e	+	o	83+89			Sibu	20
f	+	o	70+74			Walli	12
h	+	o	73+77			Jetta	6
i	+	o	75+79			Renate	8
j	+	o	79+83			Hansa	1
k	+	o	83+87			Belita	13
l	+	o	75+77			Roxy	16
o	+	o				Ulla	11
d	+	b	75+83	+	85	Vital	23
g	+	b	72+76	+	85	Premiere	26
i	+	b	75+79	+	85	Selma	7
j	+	b	79+83	+	85	Ute	9
d	+	c	75+83	+	88	Krometa	19
i	+	c	75+79	+	88	Karakter	15
j	+	c	79+83	+	88	Karolin	3
l	+	c	75+77	+	88	Sieglinde	2
k	+	d	83+87	+	92	Junior	17

6.1.1. Schematization of the banding patterns in Est 2



6.1.2. Schematization of the banding patterns in Est 3

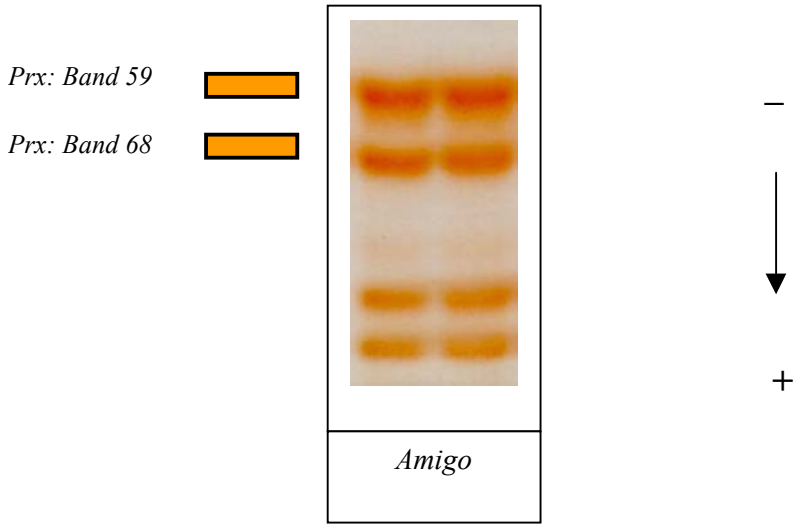


6.2. Recognition of the alleles encoding peroxidase isoenzymes

Peroxidase of the potato tuber is a monomeric enzymes. Polymorphism which should be used for the assessment of distinctness, uniformity and stability can be observed in the range REM 55 – 70. In this region 1 to 3 bands are expressed in the different varieties.

Differences in the intensity of bands are not considered as clear differences. Corresponding genotypes are scored with the same note.

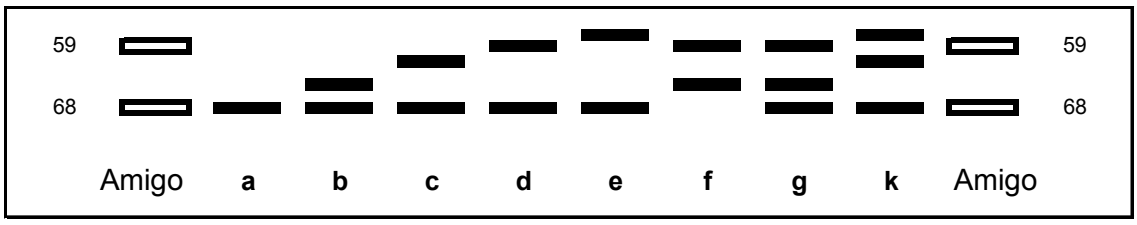
The position of the individual peroxidase isoenzymes is calibrated according to the variety Amigo. The variety Amigo has two bands in position 59 and 68.



A list of all described genotypes, example varieties and corresponding notes is provided in the following table. For schematization of band patterns see 6.2.1.

Genotype	Band Position	Example Variety	Note
a	68	Hansa	1
b	64+68	Corine	2
c	61+68	Tomensa	3
d	59+68	Amigo	4
e	58+68	Jetta	5
f	59+66	Diana	7
g	59+64+68	Thomana	6
k	58+64+68	(Scala)	-

6.2.1 Schematization of the banding patterns in Prx



6.3. Recognition of the alleles encoding patatins

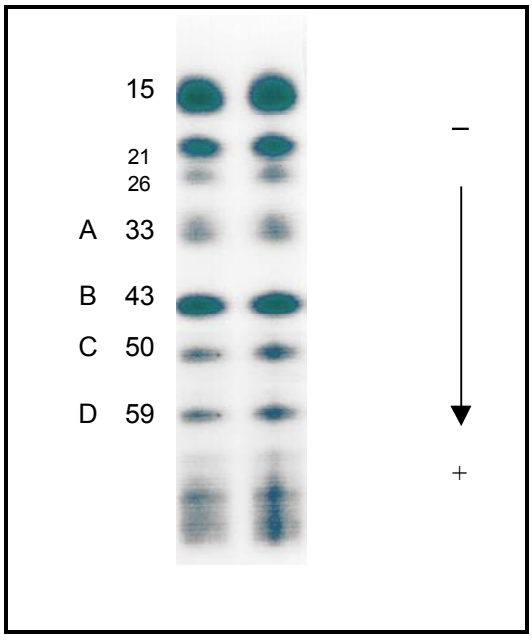
Patatin is a potato specific monomeric 40 kDa glycoprotein which is regarded as a storage protein in tubers. It is encoded by a single locus on chromosome 8 of the haploid genome representing a multigene family with 10 – 15 genes per haploid genome. The number of copies varies between varieties (BÁNFALVI et al. 1994, GANAL et al. 1991).

Patatins are highly polymorphic. More than 80 allelic combinations are known in potato varieties. The following classification is used for the denomination of the resulting banding patterns.

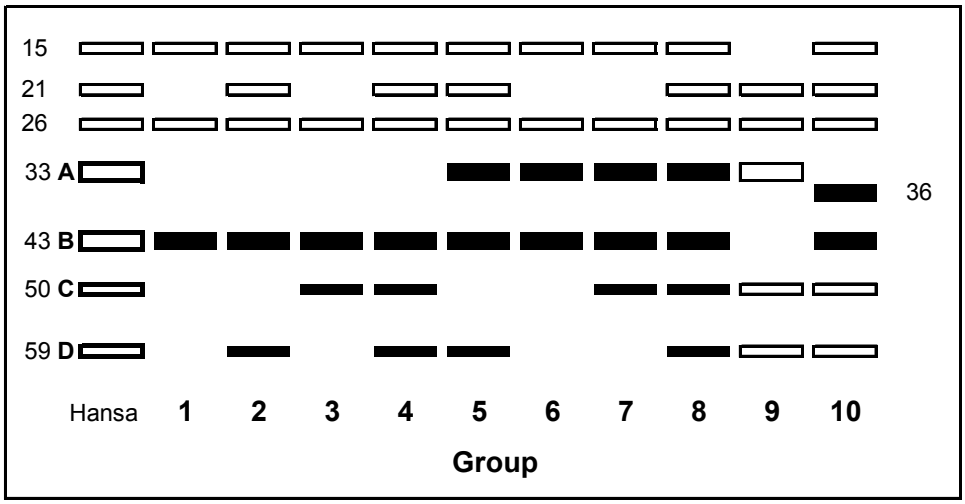
6.3.1. Classification of the banding patterns

The positions of the individual patatins is described according to their electrophoretic mobility (REM-value).

The variety Hansa is used as a standard.



All banding patterns are classified into 10 groups according to 5 prominent band regions with high mobility (REM 33 – 60). Four of these regions correspond to the A-, B-, C- and D-band groups which were used by STEGEMANN and LÖSCHKE (1976) to define group 1 - 9. Group 10 is defined by the presence of a band in position 36.



Further classification within the groups is carried out according to the exact position of the bands in the main band regions as well as the presence of bands with lower mobility.

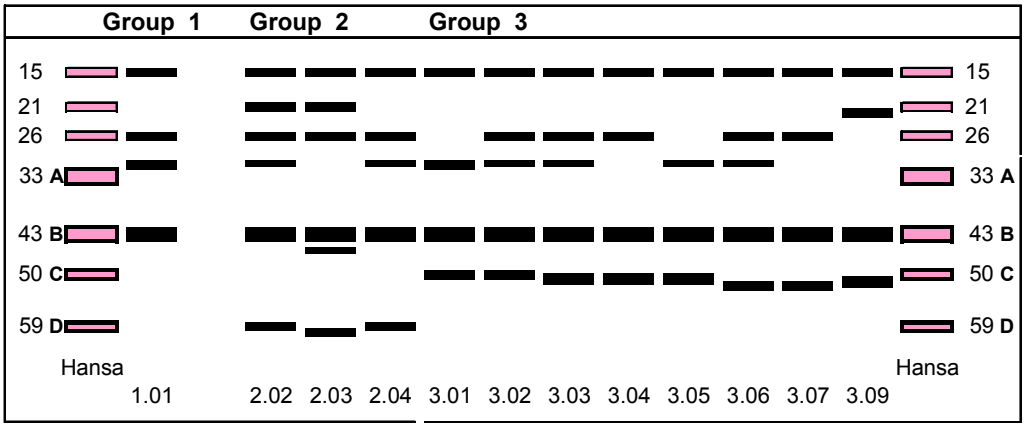
At many band positions differences in activities can be observed. Differences in band intensities are not considered as clear differences. Corresponding genotypes are scored with the same note.

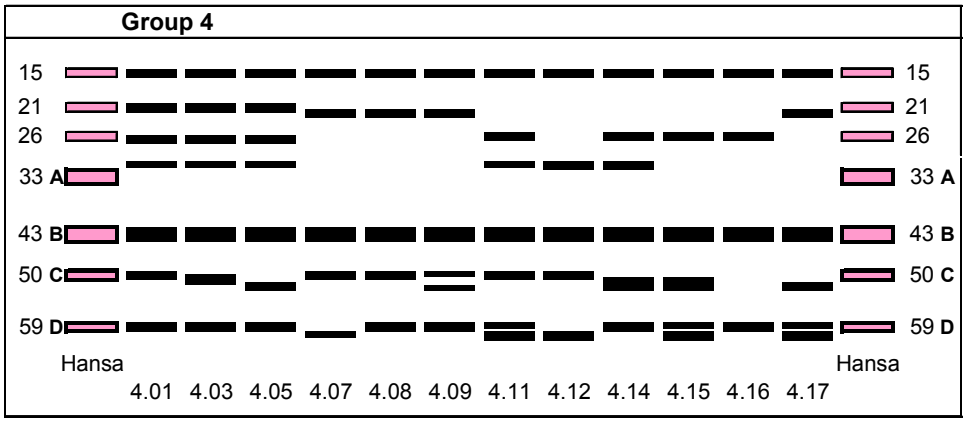
A list of all described genotypes, example varieties and corresponding notes is provided in the following table. For schematization of band patterns see 6.3.2.

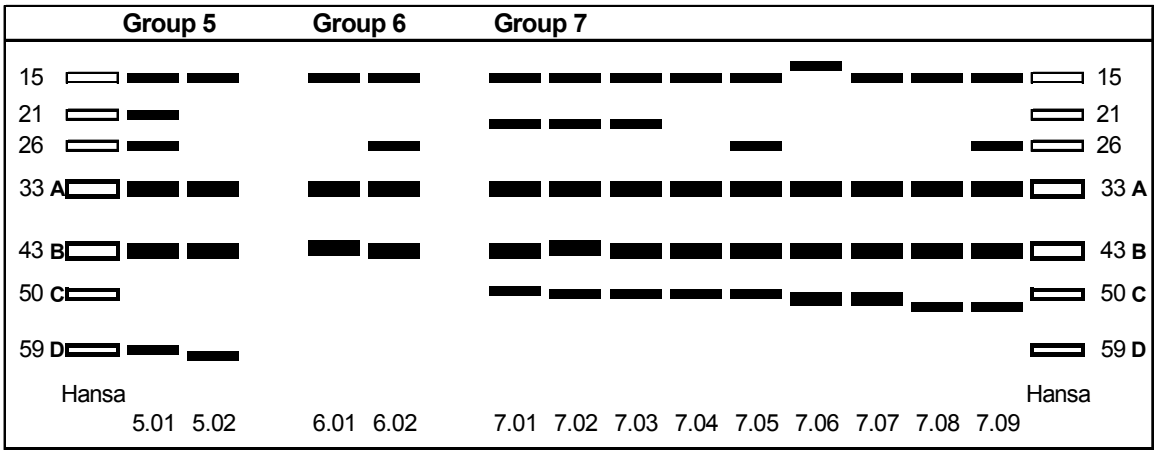
Genotype	- ➔ Band position ➔ +																		Example variety	Note
	13	15	17	21	22	26	31	33	36	42	43	46	49	50	51	52	58	59	60	
1.01		15				26	31				43									Secura	6
2.02		15		21		26	31				43							59		Desiree	11
2.03		15		21		26					43	46							60	Pompadur	77
2.04		15				26	31				43							59		Delia	16
3.01		15					31				43			50						Quarta	19
3.02		15				26	31				43			50						Irmgard	21
3.03		15				26	31				43			50	51					Ulla	47
3.04		15				26					43			50	51					Fasan	49
3.05		15					31				43			50	51					Karolin	52
3.06		15				26	31				43					52				Gloria	61
3.07		15				26					43					52				Junior	58
3.09		15			22						43			50	51					Combi	50
4.01		15		21		26	31				43			50						Indira	31
4.03		15		21		26	31				43			50	51					Pia	54
4.05		15		21		26	31				43					52				Cleopatra	65
4.07		15			22						43			50					60	Cinja	43
4.08		15			22						43			50				59		Sommergold	40
4.09		15			22						43			50		52		59		Walli	57
4.11		15				26	31				43			50				59	60	Pepo	45
4.12		15					31				43			50					60	Sarturna	42
4.14		15				26	31				43			50	51			59		Aiko	73
4.15		15				26					43			50	51			59	60	Amigo	75
4.16		15				26					43					52		59		Möwe	85
4.17		15			22						43					52		59	60	Oktan	87
5.01		15		21		26		33			43							59		Belita	13
5.02		15						33			43								60	Solina	14
6.01		15						33		42										Artana	2

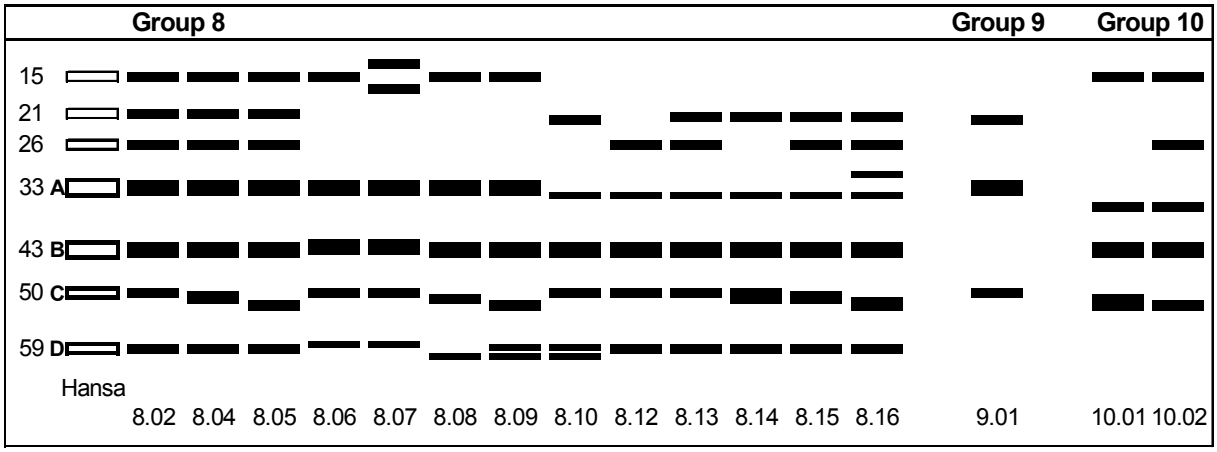
Genotype	- ➔ Band position ➔ +																				Example variety	Note
	13	**15**	**17**	**21**	**22**	**26**	**31**	**33**	**36**	**42**	**43**	**46**	**49**	**50**	**51**	**52**	**58**	**59**	**60**		
6.02		15				26		33			43										Quinta	7
7.01		15			22			33			43		49								Fausta	17
7.02		15			22			33		42				50							Orlando	86
7.03		15			22			33			43			50							Pallina	25
7.04		15						33			43			50							Grata	20
7.05		15				26		33			43			50							Atica	23
7.06	13							33			43			50	51						Karnico	4
7.07		15						33			43			50	51						Franca	51
7.08		15						33			43					52					Adretta	60
7.09		15				26		33			43					52					Ukama	62
8.02		15		21		26		33			43			50				59			Hansa	35
8.04		15		21		26		33			43			50	51			59			Shepody	55
8.05		15		21		26		33			43					52		59			Kardal	68
8.06		15						33		42				50				59			Padea	28
8.07	13		17					33			43			50			58				Elles	30
8.08		15						33			43				51	52		59			Solara	74
8.09		15						33			43						58		60		Thomana	76
8.10					22			33			43			50			58		60		Kanjer	29
8.12						26		33			43			50				59			Arnika	12
8.13				21		26		33			43			50				59			Krometa	37
8.14				21				33			43			50	51			59			Sirius	36
8.15				21		26		33			43			50	51			59			Albas	70
8.16				21		26	31	33			43			50	51			59			Feska	72
9.01					22			33						50							Calla	1
10.01		15							36		43			50	51						Liu	63
10.02		15				26			36		43					52					Kranich	80

6.3.2. Schematization of the banding patterns in PAT









Remarks to groups 5 to 8

Band 33 is an extremely broad band overlapping band 31. Therefore, in presence of band 33 band 31 is not scorable.

Literature

BÁNFALVI, Z., KOSTYÁL, Z., BARTA, E. (1994): *Solanum brevidens* possesses a non–sucrose-inducable patatin gene.
Mol. Gen. Genet. 245: 517 – 522.

GANAL, M.W., MEREDITH, W.B., ROEDER, M.S., PARK, W.D., TANKSLEY, S.D. (1991): Genetic and physical mapping of the patatin genes in potato and tomato.
Mol. Gen. Genet. 225: 501 – 509.

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

[End of Annex and of document]