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Revision of TG/80/3

TG/80/6

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

UNION INTERNATIONALE POUR LA PROTECTION DES OBTENTIONS VÉGÉTALES

INTERNATIONALER VERBAND ZUM SCHUTZ VON PFLANZEN-ZÜCHTUNGEN

UNIÓN INTERNACIONAL PARA LA PROTECCIÓN DE LAS OBTENCIONES VEGETALES

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

SOYA BEAN

(G*lycine max* (L.) Merrill)

GENEVA
1998

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Revision of TG/80/3

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(*Glycine max* (L.) Merrill.)

These Guidelines should be read in conjunction with document TG/1/2, which contains explanatory notes on the general principles on which the Guidelines have been established.

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I. Subject of these Guidelines

These Test Guidelines apply to all varieties of *Glycine max* (L.) Merill.

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 seed to be supplied by the applicant in one or several samples should be:

2 kg.

The seed should at least meet the minimum requirements for germination capacity, moisture content and purity for marketing certified seed in the country in which the application is made. The germination capacity should be as high as possible.

2. The plant material must 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 similar growing periods.

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 measurement and counting without prejudice to the observations which must be made up to the end of the growing period. Each test should include at least 300 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.

4. Additional tests for special purposes may be established.

IV. Methods and Observations

1. All observations for assessment of distinctness and stability should be made on 20 plants or parts of 20 plants

2. For the assessment of uniformity, a population standard of 0.5% with an acceptance probability of at least 95% should be applied. In the case of a sample size of 300 plants, the maximum number of off-types allowed would be 4.

3. All observations on the leaf and the flower should be made at the time of full flowering.

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. Their various states of expression should be fairly evenly distributed throughout the collection.

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

(a) Plant: color of hairs of main stem (on middle third) (characteristic 5)
(b) Flower: color (characteristic 11)
(c) Seed: hilum color (characteristic 17)
(d) Plant: time of maturity (characteristic 20)

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 (numbers), for the purposes of electronic data processing, are given opposite the states of expression for each characteristic.

3. Legend

(*) Characteristics that should be used on all varieties in every growing period over which examinations are made and always be included in the variety descriptions, 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.

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. (*)	**10**	**Hypocotyl: anthocyanin coloration**	**Hypocotyle: pigmentation anthocyanique**	**Hypokotyl: Anthocyanfärbung**	**Hipocotilo: pigmentación antociánica**		
		absent	absente	fehlend	ausente	Chandor, Goldor	1
		present	présente	vorhanden	presente	Alaric, Apache, Imari	9
2.	**10**	**Hypocotyl: intensity of anthocyanin coloration**	**Hypocotyle: intensité de la pigmentation anthocyanique**	**Hypokotyl: Intensität der Anthocyanfärbung**	**Hipocotilo: intensidad de la pigmentación antociánica**		
		very weak	très faible	sehr gering	muy débil	Azzurra	1
		weak	faible	gering	débil	Akashi, Candir	3
		medium	moyenne	mittel	media	Canton, Kendo	5
		strong	forte	stark	fuerte	Aries, Visir	7
		very strong	très forte	sehr stark	muy fuerte		9
3. (*) (+)		**Plant: growth type**	**Plante: croissance**	**Pflanze: Wuchstyp**	**Planta: crecimiento**		
		determinate	déterminée	begrenzt wachsend	determinado	Gnome, Spot, Fiskeby	1
		semi-determinate	semi-déterminée	halb begrenzt wachsend	semideterminado	Alaric, Alba, Silvia, Paradis	2
		semi-determinate to indeterminate	semi-déterminée à indéterminée	halb begrenzt wachsend bis unbegrenzt wachsend	semideterminado a indeterminado	Chandor, Kador	3
		indeterminate	indéterminée	unbegrenzt wachsend	indeterminado		4

	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
4. **(+)**	**66**	**Plant: growth habit**	**Plante: port**	**Pflanze: Wuchs-form**	**Planta: porte**		
		erect	dressé	aufrecht	erecto		1
		erect to semi-erect	dressé à demi-dressé	aufrecht bis halbaufrecht	erecto a semierecto	Tirol, Queen, Essor, Labrador	2
		semi-erect	demi-dressé	halbaufrecht	semierecto	Chandor, Apache, Paoki	3
		semi-erect to horizontal	demi-dressé à horizontal	halbaufrecht bis waagerecht	semierecto a horizontal	Alaric, Major, Sapporo	4
		horizontal	horizontal	waagerecht	horizontal		5
5. **(*)**	**65-85**	**Plant: color of hairs of main stem (on middle third)**	**Plante: couleur de la pilosité de la tige principale (au tiers central)**	**Pflanze: Farbe der Behaarung des Haupttriebes (im mittleren Drittel)**	**Planta: color de la vellosidad del tallo principal (en el tercio central)**		
		grey	grise	grau	gris	Apache, Alaric, Talon, Imari	1
		tawny	fauve	gelbbraun	castaño	Maple Glen, Chandor, Paoki, Agata	2
6. **(*)**	**85**	**Plant: height**	**Plante: hauteur**	**Pflanze: Höhe**	**Planta: altura**		
		short	basse	niedrig	baja	Carla, Paradis, Spot	3
		short to medium	basse à moyenne	niedrig bis mittel	baja a media	Trump, Essor	4
		medium	moyenne	mittel	media	Alaric, Chandor	5
		medium to tall	moyenne à haute	mittel bis hoch	media a alta	Kador	6
		tall	haute	hoch	alta	Tirol, Toréador	7

	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
7.	**65**	**Leaf: blistering**	**Feuille: cloqûre**	**Blatt: Blasigkeit**	**Hoja: abullonado**		
		absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	Bayou, Arpège, Chandor	1
		weak	faible	gering	débil	Kador, Quito	3
		medium	moyenne	mittel	medio	Paoki, Imari	5
		strong	forte	stark	fuerte	Matador	7
		very strong	très forte	sehr stark	muy fuerte		9
8. **(*)** **(+)**	**65**	**Leaf: shape of lateral leaflet**	**Feuille: forme de la foliole latérale**	**Blatt: Form der Seitenfieder**	**Hoja: forma del foliolo lateral**		
		lanceolate	lancéolée	lanzettlich	lanceolado	Toréador, Dumas, Trésor	1
		triangular	triangulaire	dreieckig	triangular	Contessa	2
		pointed ovate	pointue ovale	spitz eiförmig	oval puntiagudo	Kador, Major, Apache, Talon	3
		rounded ovate	arrondie ovale	abgerundet eiförmig	oval redondeado	Paoki, Agata, Chandor	4
9.	**65**	**Leaf: size of lateral leaflet**	**Feuille: taille de la foliole latérale**	**Blatt: Größe der Seitenfieder**	**Hoja: tamaño del foliolo lateral**		
		small	petite	klein	pequeño	Trump, Labrador, Baron, Arcade	3
		medium	moyenne	mittel	mediano	Alaric, Kushiro, Talon	5
		large	grande	groß	grande	Williams	7
10.	**65**	**Leaf: intensity of green color**	**Feuille: intensité de la couleur verte**	**Blatt: Intensität der Grünfärbung**	**Hoja: intensidad del color verde**		
		light	claire	hell	claro	Chandor, Arcade, Junior	3
		medium	moyenne	mittel	medio	Alaric, Apache, Imari	5
		dark	foncée	dunkel	oscuro	Spot. Cresir, Jedor, Ardir	7

	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
11. (*)	**66**	**Flower: color**	**Fleur: couleur**	**Blüte: Farbe**	**Flor: color**		
		white	blanche	weiß	blanca	Chandor, Crésir, Toréador	1
		violet	violette	violett	violeta	Fransoy 242, Imari, Apache, Queen	2
12.	**85**	**Pod: intensity of brown color**	**Gousse: intensité de la couleur brune**	**Hülse: Intensität der Braunfärbung**	**Vaina: intensidad del color marrón**		
		light	claire	hell	clara	Chandor, Contessa, Alba, Arcade	3
		medium	moyenne	mittel	media	Alaric, Apache, Fuji, Paoki	5
		dark	foncée	dunkel	oscura	Toréador, Tirol, Royal	7
13.	**89**	**Seed: size**	**Graine: grosseur**	**Samen: Größe**	**Semilla: tamaño**		
		small	petite	klein	pequeña	Alba, Aurélia, Flusk GT 512	3
		medium	moyenne	mittel	mediana	Queen, Goldor	5
		large	grande	groß	grande	Clédor, Cervin, Mondor	7
14.	**89**	**Seed: shape**	**Graine: forme**	**Samen: Form**	**Semilla: forma**		
		spherical	sphérique	kugelförmig	subesférica	Paoki, Valkir, Niva	1
		spherical flattened	sphérique aplatie	kugelförmig abgeflacht	subesférica aplanada	Queen, Sapporo, Clédor	2
		elongated	allongée	länglich	alargada	Soleo, Talon, Excel, Recor	3
		elongated flattened	allongée aplatie	länglich abgeflacht	alargada aplanada		4

	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
15. (*)	**89**	**Seed: ground color of testa (excluding hilum)**	**Graine: couleur de fond du tégument (à l'exclusion du hile)**	**Samen: Grundfarbe der Samenschale (ohne Nabel)**	**Semilla: color de fondo del tegumento (excluyendo el filamento)**		
		yellow	claire	gelb	amarillo	Queen, Paoki	1
		yellow green	vert jaune	gelbgrün	verde amarillento		2
		green	verte	grün	verde		3
		light brown	brun clair	hellbraun	marrón claro		4
		medium brown	brun moyen	mittelbraun	marrón medio		5
		dark brown	brun foncé	dunkelbraun	marrón oscuro		6
		black	noire	schwarz	negro		7
16. (+)	**89**	**Seed: coloration due to peroxidase activity in seed coat**	**Graine: coloration due à l'activité peroxidase dans le tégument**	**Samen: Färbung hervorgerufen durch Peroxidasereaktion in der Samenschale**	**Semillas: coloración debida a la actividad de peroxidasa en el tegumento**		
		absent	absente	fehlend	ausente	Bragg	1
		present	présente	vorhanden	presente	Hood, Hood 75	2
17. (*)	**89**	**Seed: hilum color**	**Graine: couleur du hile**	**Samen: Farbe des Nabels**	**Semilla: color del hilo**		
		grey	gris	grau	gris	Spot, Major, Apache	1
		yellow	jaune	gelb	amarillo	Maple Arrow, Imari, Talon	2
		light brown	brun clair	hellbraun	marrón claro	Kingsoy, Argenta, Baron, Opale	3
		dark brown	brun foncé	dunkelbraun	marrón oscuro	Fransoy 242, Aurélia, Léman	4
		imperfect black	noir imparfait	fast schwarz	negro imperfecto	Wells, Kador, Folio	5
		black	noir	schwarz	negro	Chandor, Queen, Paoki	6
18.	**89**	**Seed: color of hilum funicle**	**Graine: couleur de l'attache hilaire**	**Samen: Farbe des Nabelansatzes**	**Semilla: color de la inserción del hilo**		
		same as testa	même couleur que le tégument	wie Samenschale	igual que el del tegumento	Queen	1
		different to testa	couleur différente du tégument	anders als Samenschale	diferente de el del tegumento	Gieso	2

	Stage [1)] Stade [1)] Stadium [1)] Estado [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
19. (*)		**Plant: time of beginning of flowering (50% plants with at least one flower open)**	**Plante: époque de début de floraison (50 % des plantes avec au moins une fleur ouverte)**	**Pflanze: Zeitpunkt des Blühbeginns (50 % der Pflanzen mit mindestens einer geöffneten Blüte)**	**Planta: fecha del comienzo de la floración (50 % de las plantas con al menos una flor abierta)**		
		very early	très précoce	sehr früh	muy precoz	Sito, Trump, Carla, Paradis	1
		very early to early	très précoce à précoce	sehr früh bis früh	muy precoz a precoz	Labrador, Essor, Arcade	2
		early	précoce	früh	precoz	Canton, Queen, Imari	3
		early to medium	précoce à moyenne	früh bis mittel	precoz a media	Kador, Alaric, Niva	4
		medium	moyenne	mittel	media	Williams	5
		medium to late	moyenne à tardive	mittel bis spät	media a tardía		6
		late	tardive	spät	tardía		7
		late to very late	tardive à très tardive	spät bis sehr spät	tardía a muy tardía		8
		very late	très tardive	sehr spät	muy tardía		9
20. (*)	**89**	**Plant: time of maturity**	**Plante: époque de maturité**	**Pflanze: Zeitpunkt der Reife**	**Planta: fecha de la madurez**		
		very early	très précoce	sehr früh	muy precoz	Trump, Soléo, Kola, Carla, Paradis	1
		very early to early	très précoce à précoce	sehr früh bis früh	muy precoz a precoz	Chandor, Apache, Labrador	2
		early	précoce	früh	precoz	Canton, Queen, Paoki, Aurélia	3
		early to medium	précoce à moyenne	früh bis mittel	precoz a media	Kador, Kingsoy, Alaric, Niva	4
		medium	moyenne	mittel	media	Williams	5
		medium to late	moyenne à tardive	mittel bis spät	media a tardía		6
		late	tardive	spät	tardía		7
		late to very late	tardive à très tardive	spät bis sehr spät	tardía a muy tardía		8
		very late	très tardive	sehr spät	muy tardía		9

VIII. Explanation on the Table of Characteristics

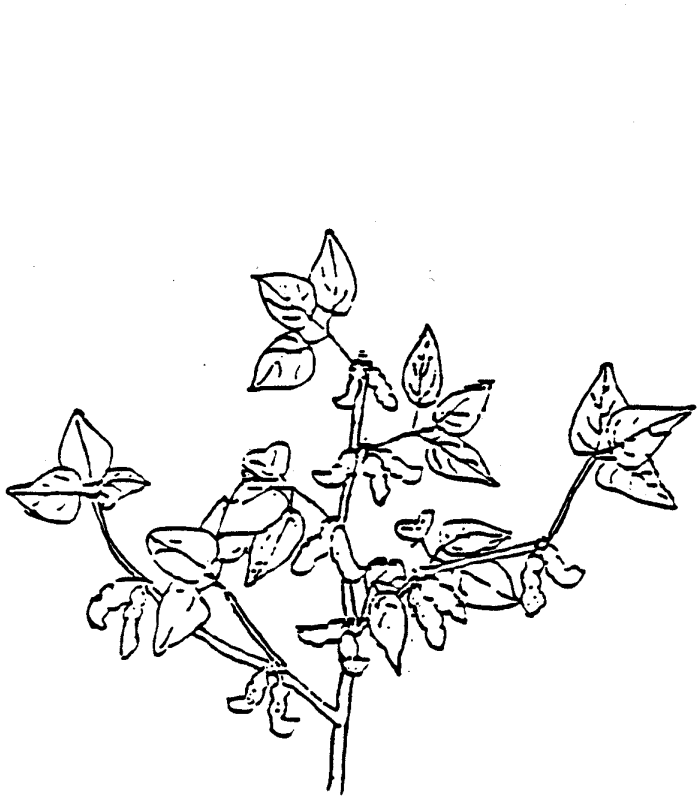
Ad. 3: Plant: growth type

– Layout: This characteristic should preferably be assessed in a special trial with 3 or 4 replicates of 20 plants each with about 9 cm between plants in the rows. Any border effect must be avoided.

– Plant material: Candidate and example varieties must be grown in groups according to their earliness at maturity (characteristic 20).

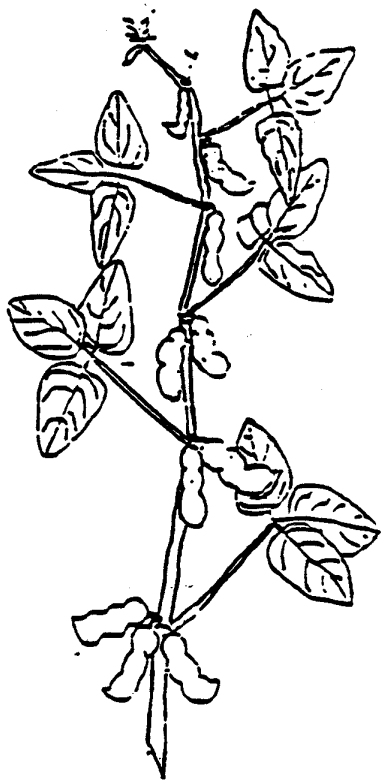
– Observation:
At the beginning of flowering time (1 flower at any level of the main stem), the apex of the plant must be identified with a mark.
At maturity (free kernels in the pod), the number of nodes between the mark and the top of the plant is counted. The average number per variety gives–in comparison with standard varieties–the state of expression of the characteristics.

In addition, the characteristic "Size of the terminal leaf" could also be considered to separate more clearly the state of expression "determinate" (Note 1) from other states. The terminal leaf on the main stem of determinate varieties is more or less equal to other leaves at lower levels. For other types, the terminal leaf is clearly smaller.



1

determinate



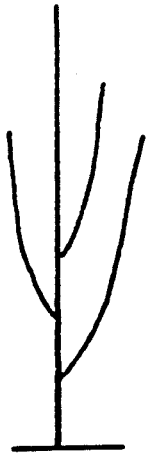
4

indeterminate

Ad. 4: Plant: growth habit

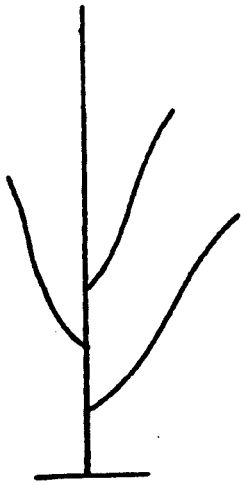
1

erect



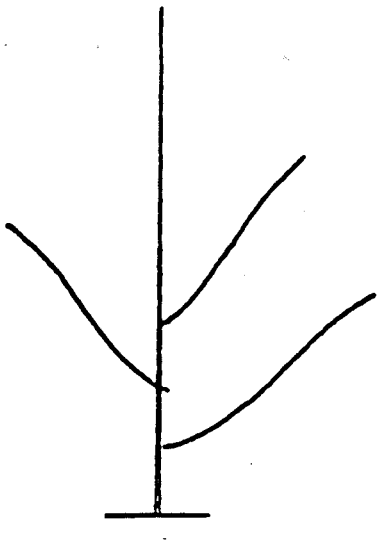
2

erect to semi-erect



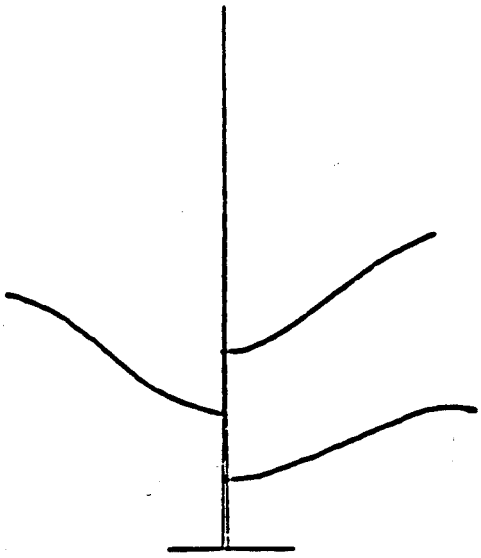
3

semi-erect



4

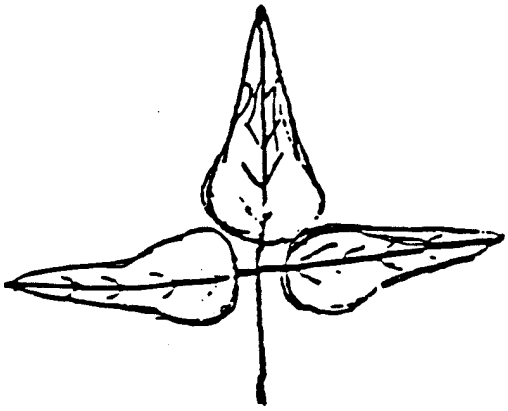
semi-erect to horizontal



5

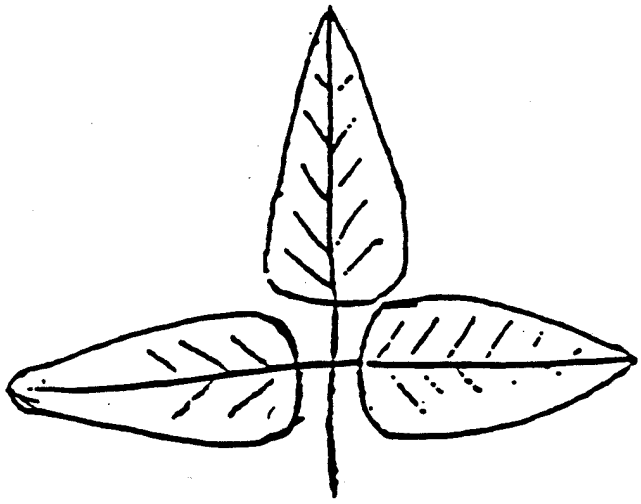
horizontal

Ad. 8: Leaf: shape of lateral leaflet



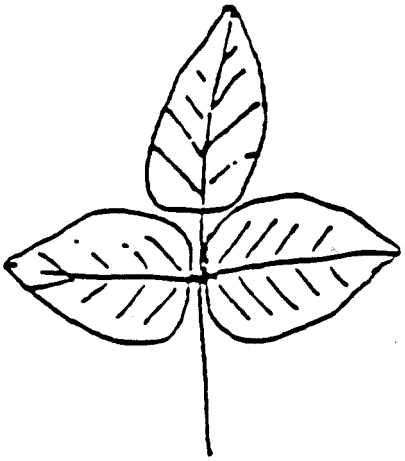
1

lanceolate



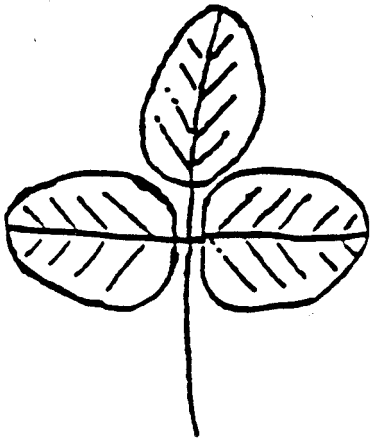
2

triangular



3

pointed ovate



4

rounded ovate

Ad. 16: Seed: coloration due to peroxidase activity in seed coat

20 seeds per variety should be tested.

The seed coat of the seed should be removed carefully so that no piece of cotyledon remains. To facilitate this procedure, the seed should be placed in water for 2 hours.

The seed coat should be placed in a cell box or in tubes (one tube per seed) and 3 to 4 cm^3 of 0,5% Guayacol solution should be added. The 0.5% Guayacol solution should be stored in the refrigerator for a period of not longer than 2 months. After having left it at room temperature for one day or more, it can no longer be used.

After 10 minutes waiting time, one drop of 0,1% H_2O_2 solution should be added.

The solution changes to dark red/brown color for a positive reaction or remains without color for a negative reaction. In order to check the 0,5% Guaycacol solution, it is advisable to include some seeds of a reference variety with a positive reaction. The recording of this reaction must be done not longer than 60 seconds after the H_2O_2 was added. It is very important that the observation must not be done longer than 60 seconds because it could lead to wrong results.

The cell box or the tubes could be softly shaken for a better reaction. For a better recording of the observation, the tubes or the cell box should be placed over a white surface.

Phenological Growth Stages and BBCH-Identification Keys of the Soybean [*]

CODE		DESCRIPTION
2- and 3 digit		
Principal growth stage 0: Germination		
00	000	Dry seed
01	001	Beginning of seed imbibition
02	002	-
03	003	Seed imbibition complete
04	004	-
05	005	Radicle emerged from seed
06	006	Elongation of radicle; formation of root hairs
07	007	Hypocotyl with cotyledons breaking through seed coat
08	008	Hypocotyl reaches the soil surface; hypocotyl arch visible
09	009	Emergence: hypocotyl with cotyledons emerged above soil surface ("cracking stage")
Principal growth stage 1: Leaf development (Main shoot)		
10	100	Cotyledons completely unfolded
11	101	First pair of true leaves unfolded (unifoliolate leaves on the first node)
12	102	Trifoliolate leaf on the 2nd node unfolded
13	103	Trifoliolate leaf on the 3rd node unfolded
1.	10.	States continuous till ....
19	109	Trifoliolate leaf on the 9th node unfolded. No side shoots visible [1]
-	110	Trifoliolate leaf on the 10th node unfolded [1]
-	111	Trifoliolate leaf on the 11th node unfolded [1]
-	112	Trifoliolate leaf on the 12th node unfolded [1]
-	113	Trifoliolate leaf on the 13th node unfolded [1]
-	11.	Stages continuous till ....
-	119	Trifoliolate leaf on the 19th node unfolded [1]

[*] Reproduced with the kind permission of the authors of: "Growth Stages of Mono- and Dicotyledonous Plants" (see Literature, Meier, Uwe (Editor), 1997)

[1] The side shoot development may occur earlier; in this case continue with the principal growth stage 2

CODE		DESCRIPTION
2- and 3 digit		
Principal growth stage 2: Formation of side shoots		
20	200	-
21	201	First side shoot visible
22	202	2nd side shoot of first order visible
23	203	3rd side shoot of first order visible
2.	20.	Stages continuous till ...
29	209	9 or more side shoots of first order visible (2 digit) 9th side shoot of first order visible (3 digit)
-	210	10th side shoot of first order visible
-	221	First side shoot of 2nd order visible
-	22.	Stages continuous till ...
-	229	9th side shoot of 2nd order visible
-	2N1	First side shoot of Nth order visible
-	2N9	9th side shoot of Nth order visible
Principal growth stage 3: [2]		
Principal growth stage 4: Development of harvestable vegetative plant parts – Main shoot -		
40	400	-
41	401	-
42	402	-
43	403	-
44	404	-
45	405	-
46	406	-
47	407	-
48	408	-
49	409	Harvestable vegetative plant parts have reached final size (Cutting of soybean plants for feeding purposes)

[2] The stem elongation of the soybean plant (Principal growth stage 3) proceeds parallel to the leaf development. Therefore a coding in the principal growth stage 3 has been omitted.

CODE		DESCRIPTION
2- and 3 digit		
Principal growth stage 5: Inflorescence emergence (Main shoot)		
50	500	-
51	501	First flower buds visible
52	502	-
53	503	-
54	504	-
55	505	First flower buds enlarged
56	506	-
57	507	-
58	508	-
59	509	First flower petals visible; flower buds still closed
Principal growth stage 6: Flowering (Main shoot)		
60	600	First flowers opened (sporadically in population)
61	601	Beginning of flowering about 10% of flowers open [3] Beginning of flowering [4]
62	602	About 20% of flowers open [3]
63	603	About 30% of flowers open [3]
64	604	About 40% of flowers open [3]
65	605	Full flowering: about 50% of flowers open [3] Main period of flowering [4]
66	606	About 60% of flowers open [3]
67	607	Flowering declining [3]
68	608	-
69	609	End of flowering: first pods visible (approximately 5 mm length) [3]

[3] This definition refers to determinate varieties
[4] This definition refers to indeterminate varieties

CODE		DESCRIPTION
2- and 3 digit		
Principal growth stage 7: Development of fruits and seeds		
70	700	First pod reached final length (15-20 mm)
71	701	About 10% of pods have reached final length (15-20 mm) [3] Beginning of pod development [4]
72	702	About 20% of pods have reached final length (15-20 mm) [3]
73	703	About 30% of pods have reached final length (15-20 mm) [3] Beginning of pod filling [4]
74	704	About 40% of pods have reached final length (15-20 mm) [3]
75	705	About 50% of pods have reached final length (15-20 mm) Continuation of pod filling.[3] Main period of pod development Continuation of pod filling [4]
76	706	-
77	707	About 70% of pods have reached final length (15-20 mm): advanced pod filling. [3] Advanced pod filling [4]
78	708	-
79	709	Approximately all pods have reached final length (15-20 mm). Seeds filling the cavity of the majority of pods [3,4]
Principal growth stage 8: Ripening of fruits and seeds		
80	800	First pod ripe, beans final color, dry and hard
81	801	Beginning of ripening; about 10% of pods are ripe, beans final color, dry and hard.[3] Beginning of pod and seed ripening [4]
82	802	About 20% of pods are ripe; beans final color, dry and hard [3]
83	803	About 30% of pods are ripe; beans final color, dry and hard [3]
84	804	About 40% of pods are ripe; beans final color, dry and hard [3]
85	805	Advanced ripening; about 50% of pods are ripe; beans final color, dry and hard.[3] Main period of pod and seed ripening [4]
86	806	About 60% of pods are ripe; beans final color, dry and hard [3]
87	807	About 70% of pods are ripe; beans final color, dry and hard [3]
88	808	About 80% of pods are ripe; beans final color, dry and hard [3]
89	809	Full maturity: approximately all pods are ripe; beans final color, dry and hard (= Harvest maturity) [3] Majority of pods are ripe; beans final color, dry and hard [4]

[3] This definition refers to determinate varieties
[4] This definition refers to indeterminate varieties

CODE 2- and 3 digit		DESCRIPTION
Principal growth stage 9: Senescence		
90	900	-
91	901	About 10% of leaves discolored or fallen
92	902	About 20% of leaves discolored or fallen
93	903	About 30% of leaves discolored or fallen
94	904	About 40% of leaves discolored or fallen
95	905	About 50% of leaves discolored or fallen
96	906	About 60% of leaves discolored or fallen
97	907	Above ground parts of plants dead
98	908	-
99	909	Harvested product (seeds)

IX. Literature

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Kiang, Y.T. and Gorman, M.B., 1985: Inheritance of NADP active isocitrate dehydrogenase isozymes in soybean. J. Hered., 76, 279-284.

Palmer, R.G., Shoemaker, R.C. and Rennie, B., 1987: Approved soybean gene symbols. Soybean Genet. Newsl., 41-58

Bourgoin-Greneche M. and Lallemand J., 1993: “L’électrophorèse et son application à la description des variétés. Présentation des techniques utilisées par le GEVES,” GEVES, France

Meier, Uwe (Editor), 1997: “Growth Stages of Mono- and Dictoyledonous Plants”, BBCH-Monograph, Blackwell Wissenschafts-Verlag Berlin-Wien 1997 (quadrilingual version: English, français, deutsch, español)

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	*Glycine max* (L.) Merrill. SOYA BEAN
2.	Applicant (Name and address)	
3.	Proposed denomination or breeder's reference	

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

4.1 Genetic origin and breeding method

(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 that question is yes, please attach a copy of such an authorization.

4.2 Other information

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 (5)	**Plant: color of hairs of main stem (on middle third; at flowering)**		
	grey	Apache, Alaric, Talon, Imari	1[ ]
	tawny	Maple Glen, Chandor, Paoki, Agata	2[ ]
5.2 (11)	**Flower: color (at full flowering)**	Chandor, Crésir, Toréador	1[ ]
	white	Fransoy 242, Imari, Apache, Queen	2[ ]
	violet		
5.3 (17)	**Seed: hilum color**		
	grey	Spot, Major, Apache	1[ ]
	yellow	Maple Arrow, Imari, Talon	2[ ]
	light brown	Kingsoy, Argenta, Baron, Opale	3[ ]
	dark brown	Fransoy 242, Aurélia, Léman	4[ ]
	imperfect black	Wells, Kador, Folio	5[ ]
	black	Chandor, Queen, Paoki	6[ ]

	Characteristics	Example Varieties	Note
5.4 (20)	**Plant: time of maturity**		
	very early	Trump, Soléo, Kola, Carla, Paradis	1[ ]
	very early to early	Chandor, Apache, Labrador	2[ ]
	early	Canton, Queen, Paoki, Aurélia	3[ ]
	early to medium	Kador, Kingsoy, Alaric, Niva	4[ ]
	medium	Williams	5[ ]
	medium to late		6[ ]
	late		7[ ]
	late to very late		8[ ]
	very late		9[ ]

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

[Annex follows]

ANNEX*

Additional Useful Explanations

TABLE OF CONTENTS PAGE

* This Annex has only been preliminarily accepted and may be amended when more information becomes available.

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.

For the analysis of enzymes, starch gel electrophoresis is recommended. Polymorphism of enzymes (i.e. 8 enzyme loci) can be detected. Genetic control is known for each enzyme locus. For the description of the method and the genetic interpretation of the zymograms, reference is made to “L’électrophorèse et son application à la description des variétés. Présentation des techniques utilisées par le GEVES,” Mireille Bourgoin-Greneche and Joëlle Lallemand, GEVES, September 1993 and additional references described in Chapter IX, Literature, of these Test Guidelines.

Part II

Characteristics Derived by Using Electrophoresis

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
21.	**Allele expression at gene locus Pgd**	**Expression allélique au locus Pgd**	**Allel-Ausprägung im Genlocus Pgd**	**Expresión del alelo en el locus Pgd**		
	Genotype	Génotype	Genotyp	Genotipo		
	a/a	a/a	a/a	a/a	Essor	1
	b/b	b/b	b/b	b/b	Apache	2
22.	**Allele expression at gene locus Idh 1 + Idh 2**	**Expression allélique au locus Idh 1 + Idh 2**	**Allel-Ausprägung in den Genloci Idh 1 + Idh 2**	**Expresión del alelo en los loci Idh 1 + Idh 2**		
	Genotype	Génotype	Genotyp	Genotipo		
	a/a + a/a	a/a + a/a	a/a + a/a	a/a + a/a	Imari	1
	a/a + b/b	a/a + b/b	a/a + b/b	a/a + b/b	Apache	2
	b/b + a/a	b/b + a/a	b/b + a/a	b/b + a/a	Essor	3
	b/b + b/b	b/b + b/b	b/b + b/b	b/b + b/b	Sapporo	4
23.	**Allele expression at gene locus Ep**	**Expression allélique au locus Ep**	**Allel-Ausprägung im Genlocus Ep**	**Expresión del alelo en el locus Ep**		
	Genotype*	Génotype*	Genotyp*	Genotipo*		
	Ep a/Ep a	Ep a/Ep a	Ep a/Ep a	Ep a/Ep a	Apache	1
	ep n/ep n	ep n/ep n	ep n/ep n	ep n/ep n	Goldor	2
24.	**Allele expression at gene locus Mpi**	**Expression allélique au locus Mpi**	**Allel-Ausprägung im Genlocus Mpi**	**Expresión del alelo en el locus Mpi**		
	Genotype	Génotype	Genotyp	Genotipo		
	b/b	b/b	b/b	b/b	Essor	1
	c/c	c/c	c/c	c/c	Apache	2

* The nomenclature used for the alleles is that approved by the Soybean Genetics Committee (PALMER *et al*, 1987). However, "n" has been added to the null alleles dia3 and ep and "a" to the active alleles Dia3 and Ep to facilitate their distinction from the denomination of the genes and to give the possibility to designate new alleles in the future.

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
25.	**Allele expression at gene locus Pgm 1**	**Expression allélique au locus Pgm 1**	**Allel-Ausprägung im Genlocus Pgm 1**	**Expresión del alelo en el locus Pgm 1**		
	Genotype	Génotype	Genotyp	Genotipo		
	a/a	a/a	a/a	a/a	Apache	1
	b/b	b/b	b/b	b/b	Essor	2
26.	**Allele expression at gene locus Acp**	**Expression allélique au locus Acp**	**Allel-Ausprägung im Genlocus Acp**	**Expresión del alelo en el locus Acp**		
	Genotype	Génotype	Genotyp	Genotipo		
	a/a	a/a	a/a	a/a	Goldor	1
	b/b	b/b	b/b	b/b	Apache	2
27.	**Allele expression at gene locus Dia 3**	**Expression allélique au locus Dia 3**	**Allel-Ausprägung im Genlocus Dia 3**	**Expresión del alelo en el locus Dia 3**		
	Genotype*	Génotype*	Genotyp*	Genotipo*		
	Dia3 a/Dia3 a	Dia3 a/Dia3 a	Dia3 a/Dia3 a	Dia3 a/Dia3 a	Apache	1
	dia3 n/dia3 n	dia3 n/dia3 n	dia3 n/dia3 n	dia3 n/dia3 n	Goldor	2

.

Part III

Description of the Method to be Used

SGE Method for Analysis of Isozymes from Soybean

1. Number of seeds for distinctness, uniformity and stability test

at least 20

2. Apparatus and equipment

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

3. Chemicals

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

3.1 Chemicals for enzyme extraction

β-mercaptoethanol
Hydrochloric acid (HC1)
Tris-(hydroxymethyl) aminomethane (Tris)

3.2 Chemicals for electrophoresis

Bromophenol blue
Citric acid monohydrate
L-Histidine
Starch hydrolyzed, for electrophoresis, (Sigma s-4501 or equivalent)

3.3 Chemicals for enzyme staining

Acetic acid glacial
Ethanol
Ethylenediamine tetra-acetic acid Na_2 salt (EDTA)
Fast Garnet GBC salt
Glucose 1-phosphate dehydrogenase (Serva 22820 or 22822 or Sigma G5885)
Hanker yates
Hydrochloric acid (HC1)
Hydrogen peroxide
DL-Isocitric acid Na_3 salt
Magnesium chloride hexahydrate
Menadoine
DL-Malic acid
Dimethylthiazol diphenyl tetrazolium (MTT)
1−Naphtylphosphate Na_3 salt dehydrate
β-Nicotinamide adenine dinucleotide (NAD)
β-Nicotinamide adenine dinucleotide reduced (NADH)
β-Nicotinamide adenine dinucleotide phosphate (NADP)
Nitro-blue tetrazolium (NBT)
6-phosphogluconic acid Na_3 salt dihydrate
Phenazine methosulfate (PMS)
Polyvinylpyrrolidone 40 (PVP-40)
Sodium acetate trihydrate
Sodium hydroxide (NaOH)
Tris-(hydroxymethyl) aminomethane (Tris)

4. Solutions

4.1 Extraction solution

10 mlTris-HCl pH 7.5 (4.3.1.3)
+ 20µl β-mercaptoethanol
made up to 100 ml with de-ionised water

4.2 Electrophoresis buffers

4.2.1 Stock solution: 0.364 M L-histidine-citrate
50.44 g L-histidine
8.20 g Citric acid monohydrate
made up to 1 l with de-ionised water

4.2.2 Running buffer: 0.072 M L-histidine-citrate pH 6.5
(Stock solution diluted 1 in 5)
400 ml stock solution (4.2.1) made up to 2 l with de-ionised water

4.2.3 Gel buffer: 0.024 M L-histidine-citrate
(Stock solution diluted 1 in 15)
80 ml stock solution (4.2.1) made up to 1200 ml with de-ionised water

4.2.4 Bromophenol blue solution
50 mg bromophenol blue dissolved in 100 ml de-ionised water

4.3 Staining solutions

4.3.1 Stock solutions

4.3.1.1 1 M Tris-HCl pH 8.0
121.1 g Tris, made up to 1 l with de-ionised water and adjusted to pH 8.0 with 50 % HCl

4.3.1.2 1 M Tris-HCl pH 9
121.1 g Tris, made up to 1 l with de-ionised water and adjusted to pH 9 with 50 % HCl

4.3.1.3 1 M Tris-HCl pH 7.5
121.1 g Tris, made up to 1 l with de-ionised water and adjusted to pH 7.5 with 50 % HCl

4.3.1.4 MTT solution
1.0 g MTT made up to 100 ml with de-ionised water

4.3.1.5 NBT solution
1.0 g NBT made up to 100 ml with de-ionised water

4.3.1.6 PMS solution
200 mg PMS, made up to 100 ml with de-ionised water

4.3.1.7 $MgCl_2$ solution
21.35 g Magnesium chloride hexahydrate
made up to 100 ml with de-ionised water

4.3.1.8 1 M Sodium acetate pH 5.5
136.08 g Sodium acetate trihydrate, made up to 1 l with de-ionised water adjusted to pH 5.5 with acetic acid glacial

4.3.2 Staining solutions (volume: 200 ml)

4.3.2.1 PGD + IDH staining solution
20 ml Tris-HCl pH 8.0 (4.3.1.1.)
+ 180 ml de-ionised water
+ 100 mg DL-Isocitric acid Na_3 salt
+ 100 mg 6-phosphogluconic acid Na_3 salt dihydrate
+ 10 ml $MgCl_2$ solution (4.3.1.7.)
+ 6 mg NADP
+ 4 ml MTT solution (4.3.1.4.)
+ 4 ml PMS solution (4.3.1.6.)

4.3.2.2 PRX staining solution
40 ml Tris HCl pH 9,0 (4.3.1.2.)
+ 160 ml de-ionised water
+ 34 µl H_2O_2
+ 200 mg Hanker yates

4.3.2.3 MPI staining solution

16 ml Tris-HCl pH 7.5 (4.3.1.3)
+ 72 ml de-ionised water
+ 48 mg Mannose 6-phosphate Na_2 salt
+ 1.6 ml $MgCl_2$ solution (4.3.1.7)
+ 20 mg NADP
+ 2 ml MTT solution (4.3.1.4)
+ 10 ml PMS solution (4.3.1.6)
+ 60 units Glucose 6-phosphate dehydrogenase
+ 120 units Phospho glucose isomerase
+ 100 ml 2% agar

4.3.2.4 PGM staining solution

20 ml Tris-HCl pH 8.0 (4.3.1.1)
+ 180 ml de-ionised water
+ 200 mg Glucose 1 phosphate
+ 20 ml $MgCl_2$ solution (4.3.1.7)
+ 10 mg NADP
+ 3 ml MTT solution (4.3.1.4)
+ 2 ml PMS solution (4.3.1.6)
+ 120 units Glucose 6-phosphate dehydrogenase

4.3.2.5 ACP staining solution

40 ml Sodium acetate pH 5.5 (4.3.1.8)
+ 160 ml de-ionised water
+ 150 mg Fast Garnet GBC salt
+ 200 mg 1-Naphthylphosphate Na_3 salt dihydrate
+ 0.5 ml $MgCl_2$ solution (4.3.1.7)

4.3.2.6 DIA staining solution

10 ml Tris-HCl pH 7.5 (4.3.1.1)
+ 190 ml de-ionised water
+ 68 mg NADH
+ 2.7 ml NBT solution (4.3.1.5)
+ 53 mg Menadione

5. Procedure

5.1 Enzyme extraction

Individual seeds are crushed with a hammer and added to 1 ml extraction buffer (4.1) at 4°C.

5.2 Preparation of the gels

To make two 12.5 % starch gels (18 x 18 x 1 cm) the following is required:
128 g starch are mixed in 1020 ml gel buffer in a 1000 ml Buchner flask at 80 ° C. The mixture is degassed for 40 seconds. The gels are poured into gel moulds as described in the user's manual of the equipment used. The formation of air bubbles should be avoided. The gels are allowed to cool at room temperature, for at least two hours, and wrapped with polyethylene film for overnight storage. Before electrophoresis, the gels are cooled at 4 ° C for at least one hour.

5.3. Electrophoresis

5.3.1 The tanks are filled with the appropriate volume of running buffer pre-cooled to 4 ° C. A slit is cut in the gel at 1 cm from the cathode. The enzyme extracts from 5.1 (30 extracts for on 18 x 18 x 1 cm gel) are absorbed onto 15 x 2 x 1 mm wicks at from Whatman n° 3 chromatography paper. The wicks are placed into the slit. At 1 cm of each edge of the gels, a wick soaked with bromophenol blue solution is inserted. The electrophoresis is carried out at 4 ° C. A constant voltage of 200 V (maximum current of 150 mA for two 18 x 18 x 1 cm gels) is applied for 20 minutes. The wicks are then removed and the electrophoresis is continued at a constant voltage of 280 V (maximum current of 180 mA for two 18 x 18 x 1 cm gels), until the bromophenol blue marker has migrated 13 cm (about 4 hours).

5.4 Enzyme staining

After electrophoresis the gel is cut horizontally in 1 mm thick slices. The upper slice is discarded. Individual gel slices are stained by incubation in the following solutions at 37 ° C in darkness:

for PGD and IDH: solution 4.3.2.1, for PRX: solution 4.3.2.2,
for MPI: solution 4.3.2.3, for PGM: solution 4.3.2.4,
for ACP: solution 4.3.2.5, for DIA: solution 4.3.2.6

The staining times range between 30 and 120 minutes. After staining the gel slices are rinsed in distilled water before being stored. The following procedures for long time-storing can be successfully used: e.g. drying of the gels between two cellophane sheets, or storing in sealed polyethylen bags.

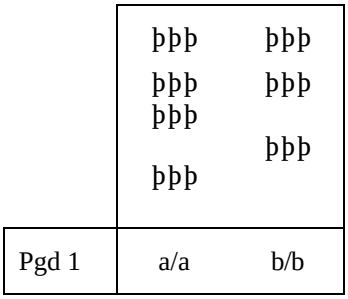
6. Recognition of the alleles encoding isoenzymes

6.1 Recognition of the alleles encoding PGD

6.1.1 Genetic interpretation of the zymogrammes

Enzyme	**Quaternary structure**	**Locus**	**Alleles**
Phosphoglucose dehydrogenase (PGD)	Dimeric	Pgd	a (Essor) b (Apache)

6.1.2 Schematization of the zymogrammes



6.2 Recognition of the alleles encoding IDH

6.2.1 Genetic interpretation of the zymogrammes

Enzyme	**Quaternary structure**	**Locus**	**Alleles**	
Isocitrate dehydrogenase (IDH)	Dimeric	Idh1	a (Apache) b (Essor)	intergenic interactions
		Idh2	a (Essor) b (Apache)	

There are interactions between the products of the genes (polypeptide subunits) encoded by Idh1 and Idh2. It is easier to analyse the two genes in combination:

Genotype		Example varieties
Idh1	*Idh2*	
b/b	a/a	Essor
a/a	a/a	Imari
b/b	b/b	Sapporo
a/a	b/b	Apache

6.2.2 Schematization of the zymogrammes

	þþþ	þþþ þþþ þþþ	þþþ þþþ þþþ	þþþ þþþ þþþ
Idh1	b/b	a/a	b/b	a/a
Idh2	a/a	a/a	b/b	b/b

6.3 Recognition of the alleles encoding PRX

6.3.1 Genetic interpretation of the zymogrammes

Enzyme	**Quaternary structure**	**Locus**	**Alleles**
Peroxydase (PRX)	Dimeric	Ep	Ep a (Apache) ep n (Goldor)

6.3.2 Schematization of the zymogrammes

	þþþ
Ep	Ep a/Ep a ep n/ep n

6.4 Recognition of the alleles encoding MPI

6.4.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Locus	Alleles
Mannose phosphate dehydrogenase (MPI)	Dimeric	Mpi	b (Essor) c (Apache)

6.4.2 Schematization of the zymogrammes

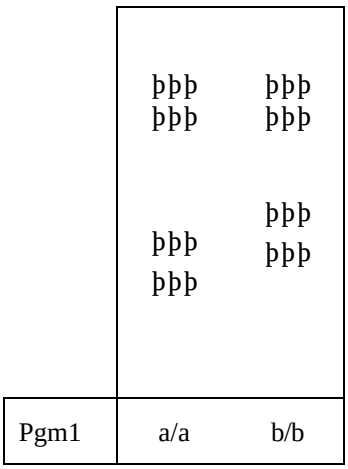
	þþþ þþþ þþþ þþþ þþþ þþþ þþþ þþþ
Mpi	b/b c/c

6.5 Recognition of the alleles encoding PGM

6.5.1 Genetic interpretation of the zymogrammes

Enzyme	Quaternary structure	Locus	Alleles
Phosphoglucomutase (PGM)	Monomeric	Pgm1	a (Apache) b (Essor)

6.5.2 Schematization of the zymogrammes



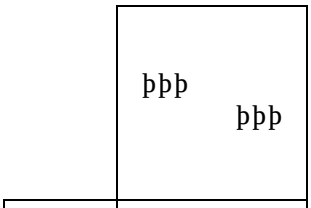
Pgm1	a/a	b/b

6.6 Recognition of the alleles encoding ACP

6.6.1 Genetic interpretation of the zymogrammes

Enzyme	**Quaternary structure**	**Locus**	**Alleles**
Acid phosphatase (ACP)	Dimeric	Acp	a (Goldor) b (Apache)

6.6.2 Schematization of the zymogrammes



Acp	a/a	b/b

6.7 Recognition of the alleles encoding DIA

6.7.1 Genetic interpretation of the zymogrammes

Enzyme	**Quaternary structure**	**Locus**	**Alleles**	
Diaphorase (DIA)	Tetrameric	Dia3	Dia3 a (Apache) dia3 n (Goldor)	intergenic interactions

There are intergenic interaction between Dia3 and Dia4

6.7.2 Schematization of the zymogrammes

Dia 3	Dia3 a/ Dia3 a	dia3 n/ dia3 n

[End of document]