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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL COMMITTEE

Thirty-Fourth Session
Geneva, March 30 to April 1, 1998

AMENDMENT TO THE DRAFT TEST GUIDELINES FOR SOYA BEAN
(TG/80/5(proj.))

prepared by experts from France in agreement with experts from Germany apart from the proposal for the system MPI

The following pages have been agreed upon between experts from France and Germany (apart from the proposal for the system MPI which the German experts do not approve but which the French experts consider to be very discriminative). They should replace the page on the literature and the Annex of document TG/80/5(proj.).

IX. Literature

Buzzell and Buttery, 1969: Inheritance of peroxidase activity on soybean seed coats. Crop Sci., 9, 387-388.

Cardy, B.J. and Beversdorf, W.D., 1984: Identification of soybean cultivars using isoenzyme electrophoresis. Seed Sci. Technol., 12 (3), 943-954.

Gorman, M.B. and Kiang, Y.T., 1977: Variety specific electrophoretic variants of four soybean enzymes. Crop Sci., 17 (6), 963-965.

Gorman, M.B. and Kiang, Y.T., 1983: Inheritance of soybean electrophoretic variants. Soybean Genet. Newsl., 10, 67-84.

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

ANNEX

Additional Useful Explanations

TABLE OF CONTENTS PAGE

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**	**Allel-Ausprägung im Genlocus Idh 1**	**Expresión del alelo en el locus Idh 1**		
	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 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
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

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
25.	**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/Ep	Ep/Ep	Ep/Ep	Ep/Ep	Apache	1
	ep-n/ep-n*	ep-n/ep-n*	ep-n/ep-n*	ep-n/ep-n*	Goldor	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/Dia3	Dia3/Dia3	Dia3/Dia3	Dia3/Dia3	Apache	1
	dia3-n/dia3-n*	dia3-n/dia3-n*	dia3-n/dia3-n*	dia3-n/dia3-n*	Goldor	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 to facilitate their distinction from the dominant alleles Ep and Dia3 and to enable computer analysis of the data

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 (HCl)
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)
Hydrochloric acid (HCl)
DL-Isocitric acid Na_3 salt
Magnesium chloride hexahydrate
DL-Malic acid
Dimethylthiazol diphenyl tetrazolium (MTT)
β-Nicotinamide adenine dinucleotide (NAD)
β-Nicotinamide adenine dinucleotide reduced (NADH)
β-Nicotinamide adenine dinucleotide phosphate (NADP)
Nitro-blue tetrazolium (NBT)
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 (4.2.1.1) 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 (4.2.1.1) 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

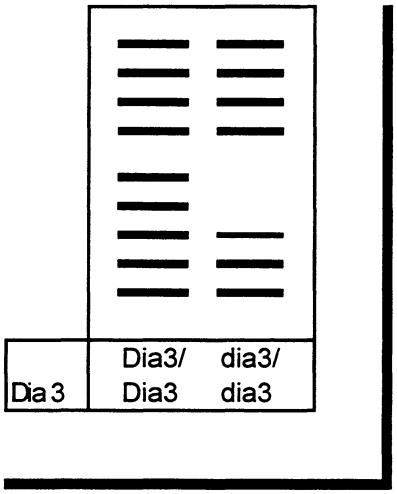
4.3.1.4 MTT solution
1.0 g MTT made up to 100 ml with de-ionised water

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

6.7.2 Schematization of the zymogrammes



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