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TG/166/3

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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

OPIUM/SEED POPPY

(*Papaver somniferum* L.)

GENEVA
1999

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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 *Papaver somniferum* L. excluding ornamental varieties.

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:

100 g.

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 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 a total of 200 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. Unless otherwise indicated, all observations determined by measurement, weighting or counting should be made on 40 plants or parts taken from each of 40 plants.

2. For the assessment of uniformity a population standard of 2% with an acceptance probability of at least 95% should be applied. In the case of a sample size of 200 plants, the maximum number of off-types allowed would be 7.

3. All observations of the stem should be made on the main stem of single spaced plants.

4. All observations of the capsule should be made on the capsule of the main stem.

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) Petal: color (characteristic 11)
(b) Petal: color of blotch (characteristic 15)
(c) Capsule: dehiscence (characteristic 26)
(d) Seed: color (characteristic 31)

VI. Characteristics and Symbols

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

2. Notes (numbers) for the purpose 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 the 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] Estadio [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1.	2	**Plant: diameter of rosette**	**Plante: diamètre de la rosette**	**Pflanze: Durchmesser der Rosette**	**Planta: diámetro de la roseta**		
		small	petit	klein	pequeño	Kompolti törpe	3
		medium	moyen	mittel	mediano	Edel-Rot, Marianne	5
		large	grand	groß	grande		7
2. **(*)**	2	**Rosette leaf: hairiness**	**Feuille de la rosette: pilosité**	**Rosettenblatt: Behaarung**	**Hoja de la roseta: vellosidad**		
		absent	absente	fehlend	ausente	Cote d'or	1
		present	présente	vorhanden	presente	Marianne	9
3. **(*)**	2	**Rosette leaf: white spots**	**Feuille de la rosette: taches blanches**	**Rosettenblatt: weiße Flecken**	**Hoja de la roseta: manchas blancas**		
		absent	absentes	fehlend	ausentes	Marianne	1
		present	présentes	vorhanden	presentes		9
4. **(*)** **(+)**	6	**Plant: branching of stem**	**Plante: ramification de la tige**	**Pflanze: Verzweigung des Triebes**	**Planta: ramificación del tallo**		
		primary	primaire	primär	primaria	Kompolti törpe	1
		secondary	secondaire	sekundär	secundaria	Edel-Rot	2
		tertiary	tertiaire	tertiär	terciaria		3
5. **(+)**	6	**Stem: length**	**Tige: longueur**	**Stengel: Länge**	**Tallo: longitud**		
		very short	très courte	sehr kurz	muy corto	Kompolti törpe	1
		short	courte	kurz	corto		3
		medium	moyenne	mittel	medio	Monako	5
		long	longue	lang	largo		7
		very long	très longue	sehr lang	muy largo	Niebieski	9

	Stage [1)] Stade [1)] Stadium [1)] Estadio [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
6. (*)	5	**Stem: anthocyanin coloration (between capsule and upper stem leaf)**	**Tige: pigmentation anthocyanique (entre la capsule et la feuille la plus haute de la tige)**	**Stengel: Anthocyanfärbung (zwischen der Kapsel und dem obersten Stengelblatt)**	**Tallo: pigmentación antociánica (entre la cápsula y la hoja más alta del tallo)**		
		absent	absente	fehlend	ausente	Kozmosz, Marianne	1
		present	présente	vorhanden	presente	Kompolti törpe	9
7.	4	**Stem: hairiness (as for 6)**	**Tige: pilosité (comme pour 6)**	**Stengel: Behaarung (wie unter 6)**	**Tallo: vellosidad (como para 6)**		
		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Santa flora	1
		weak	faible	gering	débil	Kompolti törpe	3
		medium	moyenne	mittel	media		5
		strong	forte	stark	fuerte	Edel-Weiss	7
		very strong	très forte	sehr stark	muy fuerte	Edel-Rot	9
8.	3	**Stem leaf: hue of green color (upper side)**	**Feuille sur la tige: teinte de la couleur verte (partie supérieure)**	**Stengelblatt: Ton der grünen Farbe (Oberseite)**	**Hoja del tallo: matiz del color verde (haz)**		
		absent	absente	fehlend	ausente	Magik	1
		yellowish	jaunâtre	gelblich	amarillento	Rosemarie	2
		bluish	bleuâtre	bläulich	azulado	Kozmosz	3
9.	3	**Stem leaf: waxiness**	**Feuille sur la tige: glaucescence**	**Stengelblatt: Bereifung**	**Hoja del tallo: cerosidad**		
		absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil		1
		weak	faible	gering	débil	Rosemarie	3
		medium	moyenne	mittel	media	Edel-Weiss	5
		strong	forte	stark	fuerte		7
		very strong	très forte	sehr stark	muy fuerte	Kozmosz	9

	Stage [1)] Stade [1)] Stadium [1)] Estadio [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
10. **(*)** **(+)**	3	**Stem leaf: type of incisions of margin**	**Feuille sur la tige: type d'incisions du bord**	**Stengelblatt: Typ der Randein-schnitte**	**Hoja del tallo: tipo de incisiones del borde**		
		serrate	dentelées	gesägt	serradas	Monako	1
		biserrate	en double scie	doppelt gesägt	biserradas	Keops	2
11. **(*)**	4	**Petal: color**	**Pétale: couleur**	**Blütenblatt: Farbe**	**Pétalo: color**		
		white	blanc	weiß	blanco	Marianne	1
		pink	rose	rosa	rosa	Rosemarie	2
		red	rouge	rot	rojo	Edel-Rot	3
		violet	violet	violett	violeta	Kozmosz	4
12.	4	**Petal: intensity of color (white varieties excluded)**	**Pétale: intensité de la couleur (variétés blanches exclues)**	**Blütenblatt: Intensität der Farbe (ohne weiße Sorten)**	**Pétalo: intensidad del color (excluidas las variedades blancas)**		
		light	claire	hell	claro		3
		medium	moyenne	mittel	medio		5
		dark	foncée	dunkel	oscuro		7
13. **(*)**	4	**Petal: blotch**	**Pétale: tache**	**Blütenblatt: Typ des Flecks**	**Pétalo: mancha**		
		absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Marianne	9
14. **(+)**	4	**Petal: type of blotch**	**Pétale: type de tache**	**Blütenblatt: Typ des Flecks**	**Pétalo: tipo de mancha**		
		entire blotch	tache entière	massiver Fleck	en bloque	Marianne, Rosemarie	1
		band	en bande	Streifen	en banda		2
		radial stripes	striée rayonnante	radiale Streifen	franjas radiales		3

	Stage [1] Stade [1] Stadium [1] Estadio [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
15. (*)	4	**Petal: color of blotch**	**Pétale: couleur de la tache**	**Blütenblatt: Farbe des Flecks**	**Pétalo: color de la mancha**		
		white	blanche	weiß	blanco		1
		red	rouge	rot	rojo		2
		violet	violette	violett	violeta	Kozmosz, Marianne	3
16.	4	**Petal: intensity of violet color of blotch**	**Pétale: intensité de la couleur violette de la tache**	**Blütenblatt: Intensität der Violettfärbung des Fleckes**	**Pétalo: intensidad del color violeta de la mancha**		
		light	claire	gering	claro	Rosemarie	3
		medium	moyenne	mittel	medio	Marianne	5
		dark	foncée	dunkel	oscuro	Keops	7
17. (*)	4	**Petal: incisions**	**Pétale: incisions**	**Blütenblatt: Einschnitte**	**Pétalo: incisiones**		
		absent	absentes	fehlend	ausentes	Marianne	1
		present	présentes	vorhanden	presentes		9
18. (*) (+)	4	**Petal: type of incisions**	**Pétale: type d'incisions**	**Blütenblatt: Typ der Einschnitte**	**Pétalo: tipo de incisiones**		
		sinuate	sinueuses	gebuchtet	sinuosas		1
		serrate	dentelées	gesägt	serradas		2
		laciniate	lacérées	gelappt	laciniadas		3
19. (*)	4	**Filament: color**	**Filament: couleur**	**Staubfaden: Farbe**	**Filamento: color**		
		white	blanc	weiß	blanco	Marianne	1
		light violet	violet clair	hell violett	violeta claro		2
		blackish	presque noir	fast schwarz	negruzco	Zeno	3

	Stage [1)] Stade [1)] Stadium [1)] Estadio [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
20.	5	**Capsule: waxiness**	**Capsule: glaucescence**	**Kapsel: Bereifung**	**Cápsula: cerosidad**		
		absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	Gerlach, Opal	1
		weak	faible	gering	débil		3
		medium	moyenne	mittel	media	Edel-Rot, Edel-Weiss	5
		strong	forte	stark	fuerte		7
		very strong	très forte	sehr stark	muy fuerte	Kozmosz	9
21. **(+)**	6	**Capsule: shape of longitudinal section**	**Capsule: forme de la section longitudinale**	**Kapsel: Form des Längsschnitts**	**Cápsula: forma de la sección longitudinal**		
		flattened	aplatie	abgeflacht	aplanada		1
		rectangular	rectangulaire	rechteckig	rectangular	Kék Gemona	2
		circular	circulaire	kreisförmig	circular		3
		elliptic	elliptique	elliptisch	elíptica	Kompolti törpe, Santa flora	4
		broad elliptic	elliptique large	breit elliptisch	elíptica ancha	Monako	5
		pear shaped	forme de poire	birnförmig	forma de pera	Opal	6
22. **(*)** **(+)**	6	**Capsule: shape of base**	**Capsule: forme de la base**	**Kapsel: Form der Basis**	**Cápsula: forma de la base**		
		pointed	pointue	spitz	puntiaguda	Kompolti törpe	1
		flat	plate	flach	plana	Kék Gemona	2
		recessed	déprimée	eingesenkt	deprimida	Edel-Rot	3

	Stage [1)] Stade [1)] Stadium [1)] Estadio [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
23. **(+)**	6	**Capsule: length (from base to stigmatic disc)**	**Capsule: longueur (de la base au disque stigmatique)**	**Kapsel: Länge (von der Basis zur stigmatischen Scheibe)**	**Cápsula: longitud (de la base al disco estigmático)**		
		very short	très courte	sehr kurz	muy corta		1
		short	courte	kurz	corta	Kompolti törpe	3
		medium	moyenne	mittel	media	Edel-Rot	5
		long	longue	lang	larga		7
		very long	très longue	sehr lang	muy larga	Órias Kék	9
24.	6	**Capsule: diameter**	**Capsule: diamètre**	**Kapsel: Durchmesser**	**Cápsula: diámetro**		
		very small	très petit	sehr klein	muy pequeño	Kompolti törpe	1
		small	petit	klein	pequeño		3
		medium	moyen	mittel	medio	BC-2	5
		large	large	groß	grande		7
		very large	très large	sehr groß	muy grande	Órias Kék	9
25.	6	**Capsule: ribbing**	**Capsule: côtes**	**Kapsel: Rippung**	**Cápsula: acostillado**		
		absent or very weak	nulles ou très faibles	fehlend oder sehr gering	ausente o muy débil	Kompolti M	1
		weak	faibles	gering	débil		3
		medium	moyennes	mittel	medio	Monako	5
		strong	fortes	stark	fuerte		7
		very strong	très fortes	sehr stark	muy fuerte	Gerlach, Magik	9
26. **(*)** **(+)**	6	**Capsule: dehiscence**	**Capsule: déhiscence**	**Kapsel: Dehiszenz**	**Cápsula: dehiscencia**		
		indehiscent	indéhiscente	indehiszent	indehiscente	Kék Gemona	1
		dehiscent	déhiscente	dehiszent	dehiscente	Edel-Rot	2

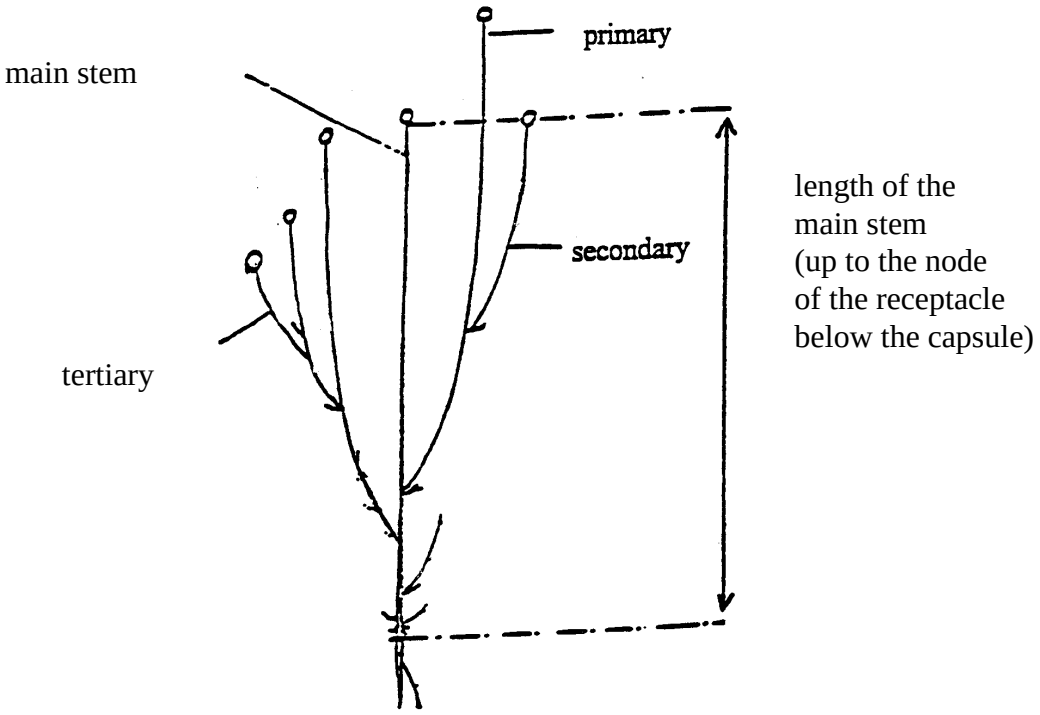
	Stage [1] Stade [1] Stadium [1] Estadio [1]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
27. **(*)** **(+)**	6	**Stigmatic disc: shape**	**Disque stigmatique: forme**	**Stigmatische Scheibe: Form**	**Disco estigmático: forma**		
		vessel-like	en vaisseau	gefäßartig	en vasija	Edel-Rot, Órias Kék	1
		dish-like	en assiette	tellerförmig	en plato		2
		flat	aplatie	flach	plano	Kompolti M	3
		conical	légèrement conique	leicht kegelförmig	ligeramente cónico	Kompolti törpe	4
		pagoda-like	en pagode	pagodenförmig	en forma de pagoda	BC-2	5
28.	6	**Stigmatic disc: number of lobes**	**Disque stigmatique: nombre de lobes**	**Stigmatische Scheibe: Anzahl Lappen**	**Disco estigmático: número de lóbulos**		
		few	faible	gering	pocos	Kompolti törpe	3
		medium	moyen	mittel	medio	Rosemarie	5
		many	grand	groß	muchos	Órias Kék	7
29. **(*)** **(+)**	6	**Stigmatic disc: surface of lobes**	**Disque stigmatique: surface des lobes**	**Stigmatische Scheibe: Oberfläche der Lappen**	**Disco estigmático: superficie de los lóbulos**		
		smooth	lisse	glatt	lisa		1
		ribbed	côtelée	gerippt	acanalada	Órias Kék	2
30. **(*)** **(+)**	6	**Stigmatic disc: apex of lobe**	**Disque stigmatique: sommet des lobes**	**Stigmatische Scheibe: Spitze der Lappen**	**Disco estigmático: ápice del lóbulo**		
		pointed	aigu	spitz	puntiagudo		1
		rounded	arrondi	abgerundet	redondeado		2
		rectangular	rectangulaire	rechteckig	rectangular	BC-2, Magik	3

	Stage [1)] Stade [1)] Stadium [1)] Estadio [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
31. (*)	6	**Seed: color**	**Semence: couleur**	**Samen: Farbe**	**Semilla: color**		
		white	blanche	weiß	blanca	Albin	1
		ochre	ocre	ockerfarben	ocre		2
		brown	brune	braun	marrón		3
		pink	rose	rosa	rosa		4
		grey	grise	grau	gris	Kompolti törpe	5
		bluish	bleuâtre	bläulich	azulada	Rosemarie	6
32.	3	**Time of flowering**	**Époque de floraison**	**Zeitpunkt der Blüte**	**Epoca de la floración**		
		very early	très précoce	sehr früh	muy temprana	Kompolti törpe	1
		early	précoce	früh	temprana	Monako	3
		medium	moyenne	mittel	media	Edel-Weiss	5
		late	tardive	spät	tardía	Lazur	7
		very late	très tardive	sehr spät	muy tardía	Libra	9
33. (+)	6	**Capsule: morphine content**	**Capsule: teneur en morphine**	**Kapsel: Morphingehalt**	**Cápsula: contenido de morfina**		
		very low	très faible	sehr gering	muy bajo	Przemko	1
		low	faible	gering	bajo	Marianne	3
		medium	moyenne	mittel	medio	Kompolti M.	5
		high	forte	stark	alto	Lomadon	7
		very high	très forte	sehr stark	muy alto		9

	Stage [1)] Stade [1)] Stadium [1)] Estadio [1)]	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
34. **(+)**	6	**Capsule: codeine content**	**Capsule: teneur en codéine**	**Kapsel: Kodeingehalt**	**Cápsula: contenido de codeína**		
		very low	très faible	sehr gering	muy bajo	Kompolti M., Marianne	1
		low	faible	gering	bajo	Lomadon	3
		medium	moyenne	mittel	medio		5
		high	forte	stark	alto	Monako	7
		very high	très forte	sehr stark	muy alto		9
35. **(+)**	6	**Capsule: thebaine content**	**Capsule: teneur en thébaïne**	**Kapsel: Thebaingehalt**	**Cápsula: contenido de tebaína**		
		none or very low	nulle ou très faible	fehlend oder sehr gering	ausente o muy bajo	Kompolti M., Marianne	1
		low	faible	gering	bajo	Monako	3
		medium	moyenne	mittel	medio	Lomadon	5
		high	forte	stark	alto		7
		very high	très forte	sehr stark	muy alto		9
36. **(+)**	6	**Capsule: narcotine content**	**Capsule: teneur en narcotine**	**Kapsel: Narkotingehalt**	**Cápsula: contenido de narcotina**		
		none or very low	nulle ou très faible	fehlend oder sehr gering	ausente o muy bajo	Kompolti M., Lomadon, Mariane	1
		low	faible	gering	bajo	Gödi N	3
		medium	moyenne	mittel	medio		5
		high	forte	stark	alto	Kék Gemona	7
		very high	très forte	sehr stark	muy alto		9

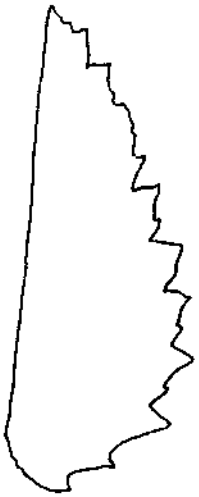
VIII. Explanations on the Table of Characteristics

Ad. 4 and 5: Plant: branching of stem (4). Stem: length (5)



primary

Ad. 10: Stem leaf: type of incisions of margin

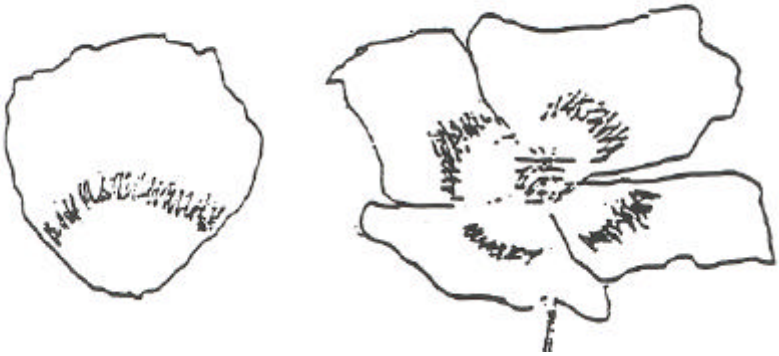


1
serrate

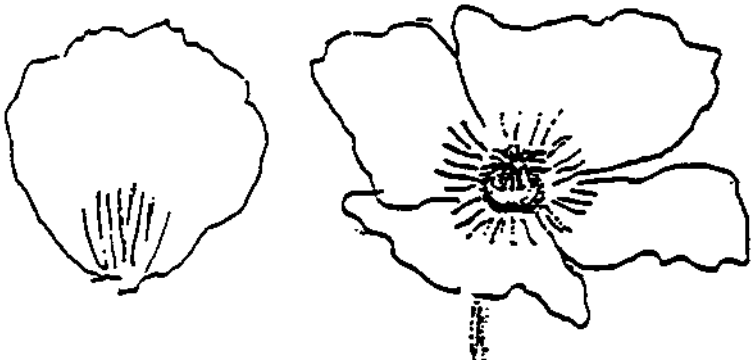
2
biserrate

Ad.14: Petal: type of blotch

1
entire blotch

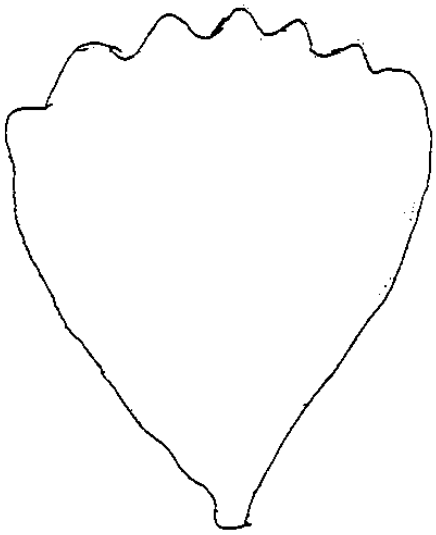


2
band

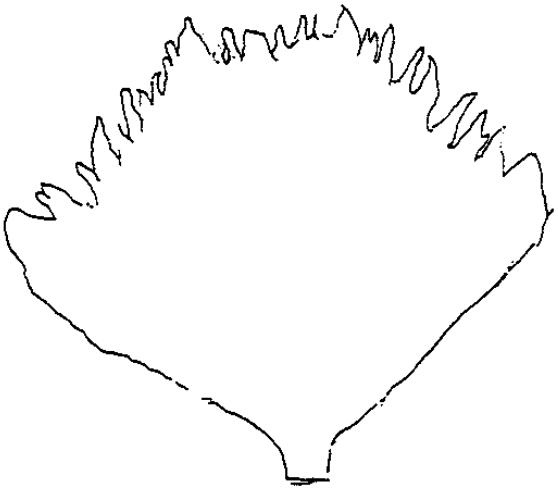


3
radial stripes

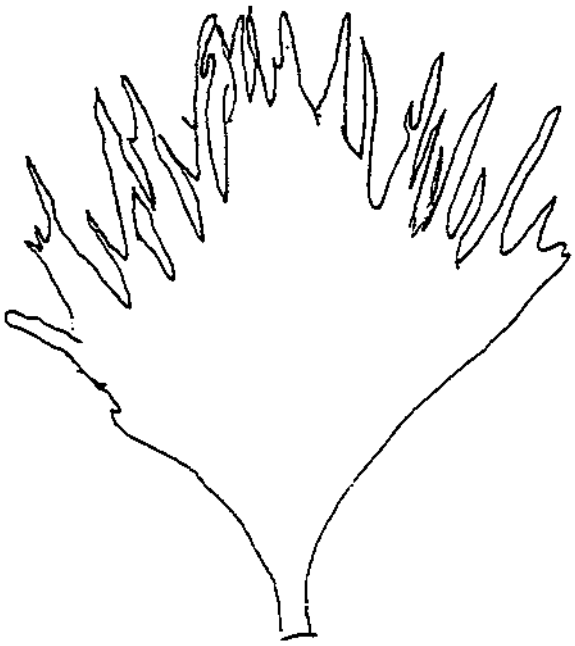
Ad. 18: Petal: type of incisions



1
sinuate



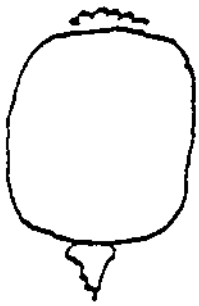
2
serrate



3
laciniate

Ad. 21: Capsule: Shape of longitudinal section

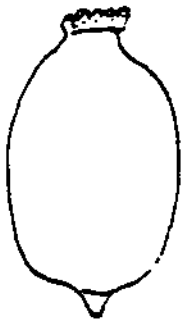
1
flattened



2
rectangular

3
circular

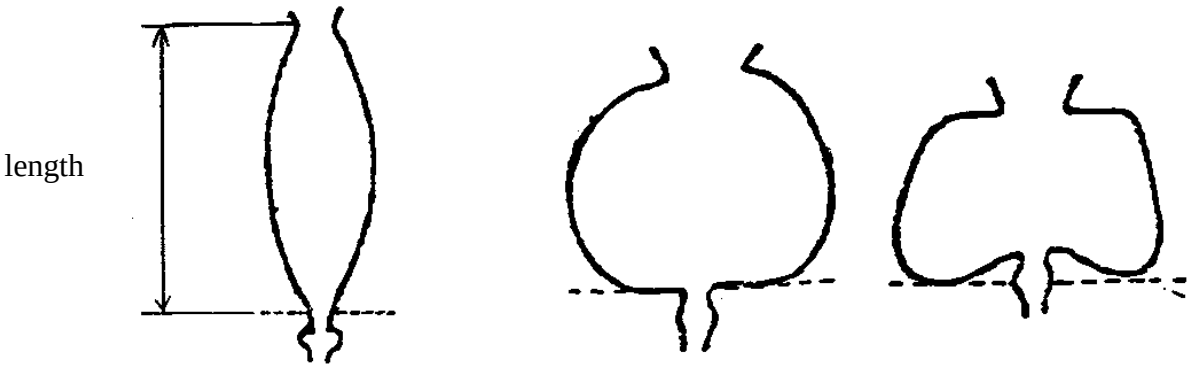
4
elliptic



5
broad elliptic

6
pear shaped

Ad. 22 and 23: Capsule: shape of base (22), length (from base to the stigmatic disc) (23)



1
pointed

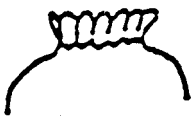
2
flat

3
recessed

Ad. 26: Capsule: dehiscence

For the observation of dehiscence the capsule should be held upside-down and shaken. If seeds do not fall out, the capsule is indehiscent (1). If seeds fall out, the capsule is dehiscent (2).

Ad. 27: Stigmatic disc: shape



1
vessel-like

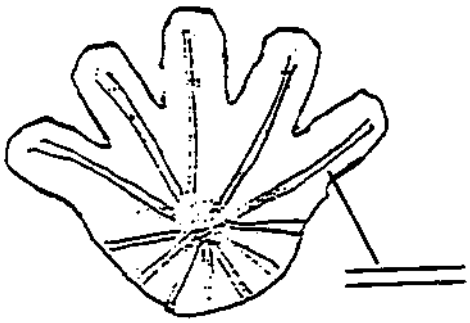
2
dish-like

3
flat

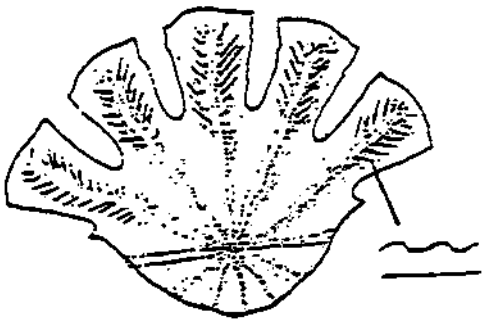
4
conical

5
pagoda-like

Ad. 29: Stigmatic disc: surface of lobes



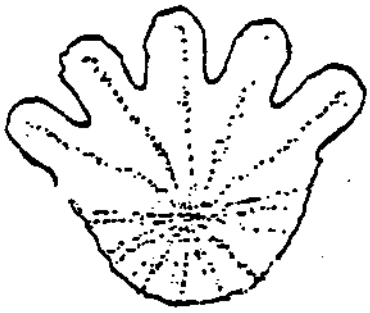
1
smooth



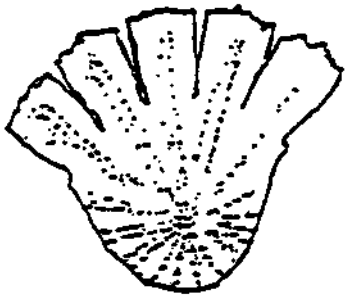
2
ribbed

Ad. 30: Stigmatic disc: apex of lobe

1
pointed



2
rounded



3
rectangular

Ad. 33-36: Capsule: determination of alkaloid contents: morphine, codeine, thebaine and narcotine

1. Sample preparation

An average sample characteristic of the variety (20 main capsules with a 10 cm stem section) should be ground to a grain size of 1-1.5 mm using a hammer mill, after which it should be homogenized to an average sample by thorough mixing.

2. Determination of morphine and codeine, thebaine and narcotine by high performance liquid chromatography (HPLC)

2.1 Preparation of the sample solution

For every variety three individual sample solutions are prepared and analyzed: Weigh 5 g of ground capsule material (1) in a 250 ml Erlenmeyer flask. Add to it 100 ml 0.1 N hydrochloric acid.

After mixing, the contents of the flask are extracted for an hour with frequent shaking, then poured into a Büchner funnel lined with filter paper and sucked into a 300 ml suction flask using a vacuum. The capsule material remaining in the funnel is returned quantitatively to the extracting flask and re-extracted for half an hour with 50 ml 0.1 N hydrochloric acid. The extract is sucked into the previous filtrate. The extracting flask is rinsed out several times with a total of 50 ml 0.1 N hydrochloric acid. The acid is poured from the flask into the filter to dissolve the drug and the washing liquid is sucked into the extract with a vacuum. The united filtrate is transferred from the suction flask to a 250 ml volumetric flask and filled to volume with 0.1 N hydrochloric acid. The sample is homogenized by shaking.

A 50 ml aliquot of the extract is pipetted into a 250 ml separatory funnel and the pH is adjusted to 9.0-9.5 with the aid of concentrated ammonia solution. The alkalized solution is then shaken with 3 x 50 ml Rasmussen mixture (chloroform: isopropanol = 3:1).

After each extraction the solvent phase is separated into a 500 ml spherical flask through a funnel, containing water-free sodium sulfate. Finally, the sodium sulfate is rinsed with a few ml of solvent mixture. The united extract is evaporated using a rotating vacuum evaporating device (e.g. Rotadest) in a water bath at 40-50^{o} C. The dry residue is dissolved in 5 or 10 ml methanol, depending on the expected morphine content of the capsule. The solution is filtered through Millipore paper with a pore size of 0.45 µm and put into ampoules.

2.2 Preparation of the standard solution

2.2.1 Standard solution (codeine, thebaine and narcotine)

Using alkaloids of known active agent content, quantities equivalent to the following anhydrous alkaloid base are weighed into a 50 ml volumetric flask:

codeine: 50.0 mg
thebaine: 10.0 mg
narcotine: 40.0 mg

The alkaloids are dissolved in methanol and the flask is filled to the mark with methanol.

2.2.2 Standard solution (morphine, codeine, thebaine and narcotine)

About 100.0 mg of morphine HCl with a known base content is weighed into a 50 ml solumetric flask and dissolved in methanol. 5.0 ml of the standard associate alkaloid solution (2.2.1) is pipetted into the solution, which is then filled to the mark with methanol.

2.3 HPLC analysis

The material is analyzed from three independent sample solutions. Each sample solution (2.1) is injected twice. Two standard solutions (2.2.2) are prepared, one of which is injected at least five times to confirm the precision of the system. The relative standard deviation calculated from the response signals of the individual peaks should have a maximum value of 1.5.

2.3.1 Equipment

Any suitable liquid chromatograph system can be used that is equipped with the following:

- adjustable wavelength UV detector
- 10-200 µl injector
- gradient programmer
- integrator

2.3.2 Conditions

Column: Lichrosorb SI-100: 5 µm, 200 mm x 4.6 mm.

Mobile phase:	A: mixture of	n-hexane:	625 ml
		methanol:	225 ml
		chloroform:	150 ml
		diethylamine:	1 ml
	B: n-hexane		

The ratio of mobile phases A and B is adjusted so that the peak resolution value between neighboring peaks is at least 1.5.

Flow rate:	2 ml/min
Column temperature:	40° C
Detection:	286 nm
Injected volume:	10 µl

2.3.3 Evaluation

The indicative retention times in case of %B=60 are the following:

narcotine:	2.1 min.
thebaine:	3.3 min.
codeine:	6.2 min.
morphine:	12.0 min.

The alkaloid contents are calculated using the following correlation:

$$C = \frac{r_m \times m_{st}}{r_{st} \times m_m} \times 1000 \quad (‰)$$

where r_m: peak area of the alkaloid in question in the sample solution
r_{st}: peak area of the alkaloid in question in the standard solution (2.2.2)
m_m: amount of capsule material in the sample solution (mg/ml)
m_{st}: concentration of the alkaloid in question in the standard solution (2.2.2) (mg/ml)

Key for the growth stages

1. Seedling (5-10 days after emergence)
2. 10-12 true leaves stage (prior to internode elongation)
3. Time of flowering (when the first flower opens on the main stem at 30% of the plants)
4. Full blossom (when the first flowers at the main stem bloom at 80% of the plants).
5. 10-14 days after the petals drop down (of primary (main) capsule).
6. Mature (dry capsule stage).

IX. Literature

Bernáth, J., Dános, B., Veres, T., Tétényi, P., 1988: "Variation and alkaloid production in poppy ecotypes: Responses to different environments." Biochemical Systematics and Ecology 16 (2): 171-178

Günther, K.F., 1975: "Beiträge zur Morphologie der Papaveraceae." Flora 164: 415-418.

Kodaira, H., and Spector, S., 1988: "Transformation of thebaine to orpavine, codeine and morphine by rat liver, kidney and brain microsomes." Proc. Natl. Acad. Sci. USA 85: 1267-1271

Hammer, K., 1981: "Probleme der Klassifikation von Papaver somniferum," Kulturpflanze 29: 287-296.

Schijfsma, L., Hoesbergen, M. and Nijdam, F.E., 1960: "A Study of the Colour and Other Characters of the Seed in Some Varieties of Oil Seed Poppy." Euphytica 9: 127-140.

ST/SOA/SER. Y./33 UN Method No. 33, Dec. 16, 1977: "Determination of Phenanthreene Alkaloids in *Papaver Somniferum* Capsules and *Papaver Bracteatum* Plant Tissue By High Performance Liquid Chromatography."

Tétényi, P., 1997: "Opium Poppy (*Papaver somniferum*) Botany and Horticulture." Horticultural Reviews, 19: 373-408

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 *Papaver somniferum* L.

OPIUM/SEED POPPY

2. Applicant (Name and address)

3. Proposed denomination or breeder's reference

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

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

	Characteristics	Example Varieties	Note
5.1 (4)	**Plant: branching of stem**		
	primary	Kompolti törpe	1[ ]
	secondary	Edel-Rot, Santa flora	2[ ]
	tertiary		3[ ]
5.2 (11)	**Petal: color**		
	white	Marianne	1[ ]
	pink	Rosemarie	2[ ]
	red	Edel-Rot	3[ ]
	violet	Kozmosz	4[ ]
5.3 (15)	**Petal: color of blotch**		
	white		1[ ]
	red		2[ ]
	violet	Kozmosz, Marianne	3[ ]
5.4 (22)	**Capsule: shape of base**		
	pointed	Kompolti törpe	1[ ]
	flat	Kék Gemona	2[ ]
	recessed	Edel-Rot	3[ ]
5.5 (26)	**Capsule: dehiscence**		
	indehiscent	Kék Gemona	1[ ]
	dehiscent	Edel-Rot	2[ ]

	Characteristics	Example Varieties	Note
5.6 (31)	**Seed: color**		
	white	Albin	1[ ]
	ochre		2[ ]
	brown		3[ ]
	pink		4[ ]
	grey	Kompolti törpe	5[ ]
	bluish	Rosemariw	6[ ]

6. Similar varieties and differences between 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

(a) Growing season:

–	spring	[ ]
–	summer	[ ]
–	autumn	[ ]
–	winter	[ ]

(b) Other conditions

.....

7.3 Capsule: morphine content (Characteristic 33)

		Example Varieties	
–	very low	Przemko	[ ]
–	low	Marianne	[ ]
–	medium	Kompolti M.	[ ]
–	high	Lomadon	[ ]
–	very high		[ ]

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

[End of document]