



EUROPEAN UNION

COMMUNITY PLANT VARIETY OFFICE

PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Secale cereale L.

RYE

UPOV Species Code: SECAL_CER

Adopted on 31/10/2002

I - SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on general UPOV Document TG/1/3 and UPOV Guideline TG/58/6 dated 4th March 1999 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to all varieties of *Secale cereale* L.

II - SUBMISSION OF SEED AND OTHER PLANT MATERIAL

1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of

- the closing date for the receipt of plant material;
- the minimum amount and quality of plant material required;
- the Examination Office to which material is to be sent.

A sub-sample of the material submitted for test will be held in the variety collection of the Examination Office as the definitive sample of the candidate variety.

The applicant is responsible for ensuring compliance with any customs and plant health requirements.

2. Final dates for receipt of documentation and material by the Examination Office

The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen.

The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO if no plant material has been received. However, if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Seed requirements

Final dates for request for technical examination and sending of Technical Questionnaire by the CPVO as well as submission date of plant material by the applicant (the seed requirements can be subject to changes, actual information is on the CPVO web site, www.cpvo.europa.eu).

Examination Office in	Request of examination	Plant material	Seed requirements
GERMANY	spring 05/01 winter 25/08	spring 15/01 winter 10/09	5 kg of seeds, Hybrids: additional 2 kg of seeds of each component incl. single cross

Cited are closing and submission dates and seed requirements for national applications

Quality of seed:The minimum requirements for germination capacity and analytical purity should not be less than the standards laid down in EC Directive 66/402/EEC

Seed Treatment:.....The plant material must not have undergone any treatment unless the CPVO and the Examination Office allow or request such treatment. If it has been treated, full details of the treatment must be given.

Labelling of sample: ...- Species
- File number of the application allocated by the CPVO
- Breeder's reference
- Examination reference (if known)
- Name of applicant
- The phrase "On request of the CPVO".

III - CONDUCT OF TESTS

1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;
- varieties protected in other UPOV Member States;
- any other variety in common knowledge.
- In case of hybrids, all components of hybrid varieties in common knowledge must be considered as part of the reference collection.

The composition of the variety collection in each Examination Office depends on the ecological conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

2. Material to be examined

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the variety collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate the work with other offices involved in DUS-testing of rye. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

3. Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted. In the latter case, the CPVO should be informed. In addition the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N° 1239/95, to insert additional characteristics and their expressions in respect of a variety.

4. Grouping of varieties

The varieties and candidates to be compared will be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups. The characteristics that could be used for grouping are the following (CPVO numbering; G for grouping in table of characteristics)

- a) Ploidy (characteristic 1)
- b) Seasonal type (characteristic 22)

5. Trial designs and growing conditions

The minimum duration of tests will normally be two independent growing cycles. Tests will be carried out under conditions ensuring normal growth. The size of the plots will be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing cycle.

As a minimum, each test should include at least:

600 plants in row plots which should be divided between two replicates and
60 single-spaced plants which should be divided between three replicates.

The assessment for the characteristic "Seasonal type" should be carried out on at least 500 plants.

In case of hybrid varieties all components which are necessary for the seed production have to be included into the test. More generally, for any application the components involved in the production of the variety must be included into the test.

6. Special tests

In accordance with Article 83(3) of Council Regulation No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

7. Standards for decisions

a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of Article 7 of Council Regulation No. 2100/94.

Qualitative characteristics:

In the case of characteristics which show discrete discontinuous states of expression, a difference between two varieties is clear if the respective characteristics have expressions which fall into two different states.

Quantitative characteristics:

Characteristics which show a continuous range of expression from one extreme to the other may be either measured or visually observed.

In the case of visually observed characteristics, a difference between two varieties is clear if the expression of the respective characteristics differs by at least the span of one note, taking into account the variability observed within the varieties.

If distinctness is assessed using the t-test least significant difference the difference between two varieties is clear if it occurs with the same sign at the 1% significance level or less ($p \leq 0.01$) in two consecutive or two out of three growing cycles.

If distinctness is assessed by the combined over years distinctness analysis (COYD) the difference between two varieties is clear if the respective characteristics are different at the 1% significance level or less ($p \leq 0.01$) in a test over either two or three years.

If the significance level or statistical methods proposed are not appropriate the method used should be clearly described.

b) **Uniformity**

Parental lines and single crosses

Uniformity is assessed by visual observation and the detection of off-types.

The assessment of uniformity should – if not otherwise indicated – be done on 600 plants. A population standard of 0.5% with an acceptance probability of at least 95% should be applied. The maximum number of off-types allowed would be 6.

Where the assessment of uniformity is carried out on a sample size of 60 plants or parts of 60 plants – if not otherwise indicated- a population standard of 2% with an acceptance probability of at least 95% should be applied. The maximum number of off-types allowed would be 3 in 60.

Open pollinated varieties and other hybrids than single crosses

For open-pollinated varieties and other hybrids than single crosses, the variability within the variety should not exceed the variability of comparable varieties already known.

If not otherwise indicated uniformity is assessed on the basis of 60 plants.

c) **Stability**

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity.

Seed samples of further submissions included in any test must show the same expression of characteristics as the material originally supplied.

IV - REPORTING OF RESULTS

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two growing cycles but in some cases three growing cycles may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive interim reports and final reports by the date agreed between the CPVO and the Examination Office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

V - LIAISON WITH THE APPLICANT

If problems arise during the course of the test the CPVO should be informed so that the information can be passed on to the applicant. Subject to prior permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

VI - TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTION

CPVO N°	UPOV N°	Characteristics	Stage, Method ¹	Examples ²	Note
1.	1.	Ploidy			
		diploid		Farino; Sorom	2
G		tetraploid		Tero	4
2.	2.	Grain: colour of aleurone layer	00		
		(+) ³ light	VG	Tetrahell	1
		dark		Pekuro; Sorom	2
3.	3.	Coleoptile: anthocyanin coloration	10-11		
		(+) absent or very weak	VG		1
		weak			3
		medium			5
		strong		Calypso; Sorom	7
very strong			9		
4.	4.	Coleoptile: length	12-13		
		(+) very short	A; MS		1
		short			3
		medium		Clou; Sorom	5
		long		Uso	7
very long			9		

¹ The optimum stage of observation is explained in Annex 1 in 'Explanations and Methods'. The sample size and the method of observation for each characteristic are indicated by numbers and letters. Explanations are given in Annex 1 in 'Explanations and Methods'

² For certain characteristics, different example varieties, separated by a semicolon, are indicated for winter rye and spring rye. Where spring rye varieties are indicated they follow the semicolon. Example varieties are given as an indication, others may be used.

³ See explanations in Annex 1 'Explanations and methods'.

CPVO N°	UPOV N°	Characteristics	Stage, Method ¹	Examples ²	Note	
5.	5.	First leaf: length of sheath	12-13			
		(+)	very short	A; MS	1	
			short		Cero	3
			medium		Clou; Sorom	5
			long		Protector	7
		very long			9	
6.	6.	First leaf: length of blade	12-13			
		(+)	very short	A; MS	1	
			short		Cero	3
			medium		Farino; Sorom	5
			long		Protector	7
		very long			9	
7.	7.	Plant: growth habit	25-29			
		(+)	erect	⁴ B; VG	1	
			semi-erect	A; VS	Protector	3
			intermediate		- ;Sorom	5
			semi-prostrate		Calypso	7
		prostrate			9	
8.	8.	Flag leaf: glaucosity of sheath	50-60			
		(+)	absent or very weak	B; VG	1	
			weak	A; VS	Protector	3
			medium		- ;Sorom	5
			strong		Amando	7
		very strong			9	

⁴ First indication always refers to sample size and method of observation for parental lines; second indication always refers to open pollinated varieties and other hybrids than single crosses.

CPVO N°	UPOV N°	Characteristics	Stage, Method ¹	Examples ²	Note
9. (+)	9.	Time of ear emergence	52		
		very early	B; MG		1
		early	A; MS	Danko	3
		medium		Farino; Sorom	5
		late			7
		very late			9
10.	10.	Leaf next to flag leaf: length of blade	60-69		
		very short	B; MS		1
		short	A; MS	Amando	3
		medium		Dino; Sorom	5
		long			7
		very long			9
11.	11.	Leaf next to flag leaf: width of blade	60-69		
		very narrow	B; MS		1
		narrow	A; MS	Amando	3
		medium		Protector; Sorom	5
		broad			7
		very broad			9
12.	12.	Ear: glaucosity	69-75		
		absent or very weak	B; VG		1
		weak	A; VS		3
		medium		- ;Sorom	5
		strong		Motto	7
		very strong			9

CPVO N°	UPOV N°	Characteristics	Stage, Method ¹	Examples ²	Note
13. (+)	13.	Stem: hairiness below ear	70-85		
		absent or very weak	B; VG	Halo; Sorom	1
		weak	A; VS	Uso; Petka	3
		medium			5
		strong			7
		very strong			9
14.	14.	Plant: length (stem; ear and awns)	80-92		
		very short	B; MS		1
		short	A; MS	Calypso	3
		medium		- ;Sorom	5
		long		Protector	7
		very long			9
15.	15.	Stem: length between upper node and ear	80-92		
		very short	B; MS		1
		short	A; MS	Calypso	3
		medium		Borellus; Sorom	5
		long		Protector	7
		very long			9
16.	16.	Ear: length (without awns)	80-92		
		very short	B; MS		1
		short	A; MS	Danko	3
		medium		Uso; Sorom	5
		long		Protector	7
		very long			9

CPVO N°	UPOV N°	Characteristics	Stage, Method ¹	Examples ²	Note
17. (+)	17.	Ear: density	80-92		
		very lax	B; MS		1
		lax	A; MS	Protector	3
		medium		Hacada; Sorom	5
		dense		Danko	7
		very dense			9
18.	18.	Ear: attitude	90-92		
		erect	B; VG		1
		semi-erect	A; VS		3
		horizontal		Calypso; Sorom	5
		semi-recurved			7
		recurved			9
19. (+)	19.	Grain: weight per thousand grains	90-92		
		very small	MG		1
		small		Rheidol	3
		medium		Danko; Sorom	5
		large			7
		very large		Clou	9
20. (+)	20.	Grain: length	92		
		very short	A, MS		1
		short		Uso	3
		medium		Esprit; Sorom	5
		long			7
		very long			9

CPVO N°	UPOV N°	Characteristics	Stage, Method ¹	Examples ²	Note
21.	21.	Grain: coloration with phenol	92		
(+)		absent or very light	VG		1
		light			3
		medium		Clou; Sorom	5
		dark		Esprit; Petka	7
		very dark			9
22.	22.	Seasonal type	B; VG		
		winter		Farino	1
		alternative			2
G		spring		- ;Sorom	3

ANNEXES TO FOLLOW

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ANNEX II

Technical Questionnaire

ANNEX I

EXPLANATIONS AND METHODS

Method of observation of characteristics

Letters indicate the relevant method for the assessment of uniformity and distinctness. In the table of characteristics the first indication refers to the sample size and the method of observation for parental lines, the second indication for open pollinated varieties and other hybrids than single crosses.

A	Sample size of 60 plants to be observed for the assessment of uniformity
B	Sample size of 600 plants to be observed for the assessment of uniformity
MG	Measurement of a group of plants or plant parts for the assessment of distinctness
MS	Measurement of single plants or plant parts for the assessment of distinctness
VG	Visual assessment by a single observation of a group of plants or plant parts for the assessment of distinctness
VS	Visual assessment by a single observation of a number of individual plants or plant parts for the assessment of distinctness

Ad. 2: Grain: colour of aleurone layer

The colour should be assessed visually on at least 100 grains of the material sent in for testing.

Ad. 3: Coleoptile: anthocyanin coloration

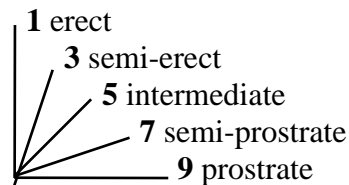
The anthocyanin coloration should be assessed visually in the laboratory. For this purpose 100 grains should be placed on filter paper and germinated on germination tables at a temperature of 15 to 16° C in darkness. When the coleoptile has reached about 1 cm in length (after 5 to 6 days); the plants should receive for 4 days without interruption light of about 13 000 to 15 000 lux at room temperature (18 - 19° C).

Ad. 4 - 6: Coleoptile: length (4); First leaf: length of sheath (5); length of blade (6)

3 x 24 grains of the material sent in for testing are sown in multipot plates with standard soil in 1 cm sowing depth. The plants are produced in the greenhouse at 20° C and with additional light for 12 hours per day during 12 days. 20 plants per replicate are measured.

Ad. 7: Plant: growth habit

The growth habit should be assessed on single-spaced plants visually from the attitude of the leaves and tillers. The angle formed by the outer leaves and the tillers with an imaginary middle axis should be used. The states of expression should be determined as follows:



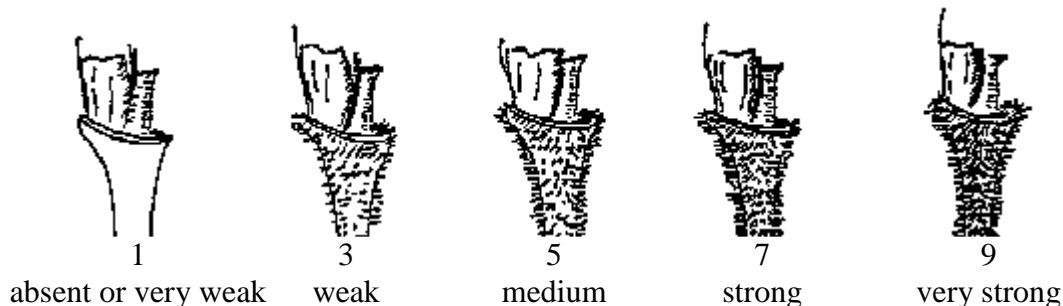
Ad. 8: Flag leaf: glaucosity of sheath

The observation should be done on the upper third of the sheath.

Ad. 9: Time of ear emergence

To assess the time; the number of plants which have reached stage 52 of the EUCARPIA Decimal Code for the Growth Stages of Cereals should be recorded at two-day intervals. From these data the average time of ear emergence of the variety should be calculated.

Ad. 13: Stem: hairiness below ear



Ad. 17: Ear: density

The density should be assessed by calculating the average number of rachis segments per length of ear.

Ad. 19 + 20: Grain: weight per thousand grains (19); length (20)

The weight and the length should be assessed by taking one harvested bunch each from the row plots. The length should be observed on 60 grains.

Ad. 21: Grain: coloration with phenol

Method for Determination of Phenol Reaction

Number of grains per test:	100 The grains should not have been treated chemically
Preparation of grains:	Soak in tap water for 16 to 20 hours; drain and remove surface water; place the grains with crease downwards; cover dish with lid
Concentration of solution:	1% Phenol-solution (freshly made up)
Amount of solution:	2 ml in a petri-dish on filter paper
Place:	Laboratory
Light:	Daylight; out of direct sunshine
Temperature:	18 to 20° C
Time of recording:	4 hours after adding solution
Scale of recording:	See characteristic 21 in the Table of Characteristics
Note:	At least two of the example varieties should be included as a control

DECIMAL CODE FOR THE GROWTH STAGE

2-digit Code	General Description	Feekes' Scale	Additional Remarks on Wheat; Barley; Rye; Oats and Rice
	<u>Germination</u>		
00	Dry seed		
01	Start of imbibition		
02	-		
03	Imbibition complete		
04	-		
05	Radicle emerged from caryopsis		
06	-		
07	Coleoptile emerged from caryopsis		
08	-		
09	Leaf just at coleoptile tip		
	<u>Seedling growth</u>		
10	First leaf through coleoptile	} 1	Second leaf visible (less than 1 cm)
11	First leaf unfolded(1)		
12	2 leaves unfolded	} } } } } } }	50% of laminae unfolded
13	3 leaves unfolded		
14	4 leaves unfolded		
15	5 leaves unfolded		
16	6 leaves unfolded		
17	7 leaves unfolded		
18	8 leaves unfolded		
19	9 or more leaves unfolded		

2-digit Code	General Description	Feekes' Scale	Additional Remarks on Wheat; Barley; Rye; Oats and Rice
<u>Germination</u>			
20	Main shoot only		
21	Main shoot and 1 tiller	2	This section to be used to supplement records from other sections of the table: "concurrent codes."
22	Main shoot and 2 tillers	}	
23	Main shoot and 3 tillers	}	
24	Main shoot and 4 tillers	}	
25	Main shoot and 5 tillers	}	
26	Main shoot and 6 tillers	3	
27	Main shoot and 7 tillers	}	
28	Main shoot and 8 tillers	}	
29	Main shoot and 9 or more tillers	}	
<u>Stem elongation</u>			
30	Pseudo stem erection (2)	4 - 5	In rice: vegetative lag phase
31	1st node detectable	6	} Jointing stage
32	2nd node detectable	7	
33	3rd node detectable	}	Above crown nodes
34	4th node detectable	}	
35	5th node detectable	}	
36	6th node detectable	}	
37	Flag leaf just visible	8	
38	-		
39	Flag leaf ligule/collar just visible	9	Pre-boot stage In rice: opposite auricle stage

2-digit Code	General Description	Feekes' Scale	Additional Remarks on Wheat; Barley; Rye; Oats and Rice
	<u>Booting</u>		
40	-		Little enlargement of the inflorescence; early-boot stage
41	Flag leaf sheath extending		
42	-		
43	Boots just visibly swollen	}	Mid-boot stage
44	-	}	10
45	Boots swollen	}	Late-boot stage
46	-		
47	Flag leaf sheath opening	}	
48	-	}	
49	First awns visible	}	In awned forms only
		10.1	
	<u>Inflorescence emergence</u>	}	
50	} First spikelet of	} N	N = non-synchronous crops
	} inflorescence just	}	
51	} visible	} S	S = synchronous crops
52	}	} N	
	} ¼ of inflorescence emerged	}	10.2
53	}	} S	
54	}	} N	
	} ½ of inflorescence emerged	}	10.3
55	}	} S	
56	}	} N	
	} ¾ of inflorescence emerged	}	10.4
57	}	} S	
58	}	} N	
	} Emergence of inflorescence	}	10.5
59	} completed	} S	

2-digit Code	General Description	Feekes' Scale	Additional Remarks on Wheat; Barley; Rye; Oats and Rice
	<u>Anthesis</u>		
60	{	{ N	
	{ Beginning of anthesis	{ 10.51	
61	{	{ S	Not easily detectable in barley.
62	-		In rice: Usually immediately following heading
63	-		
64	{	{ N	
	{ Anthesis half-way	{ 10.52	
65	{	{ S	
66	-		
67	-		
68	{	{ N	
	{ Anthesis complete	{ 10.53	
69	{	{ S	
	<u>Milk development</u>		
70	-		
71	Caryopsis watery ripe	10.54	
72	-		
73	Early milk	{	
74	-	{	
75	Medium milk	{ 11.1	{ Increase in solids of liquid endosperm
76	-	{	{ notable when crushing the caryopsis
		{	{ between fingers
77	Late milk	{	
78	-		
79	-		
	<u>Dough development</u>		
80	-		
81	-		
82	-		
83	Early dough	{	
		{	

2-digit Code	General Description	Feekes' Scale	Additional Remarks on Wheat; Barley; Rye; Oats and Rice
84	-	}	Fingernail impression not held.
85	Soft dough		11.2
86	-		.
87	Hard dough		.
88	-		Fingernail impression held; inflorescence losing chlorophyll
89	-		
	<u>Ripening</u>		
90	-		In rice: Terminal spikelets ripened.
91	Caryopsis hard (difficult to divide by thumbnail) (3)	11.3	In rice: 50% of spikelets ripened
92	Caryopsis hard (can no longer be dented by thumbnail) (4)	11.4	In rice: Over 90% of spikelets ripened (5)
93	Caryopsis loosening in daytime		Risk of grain loss by shedding
94	Over-ripe; straw dead and collapsing		
95	Seed dormant		
96	Viable seed giving 50% germination		
97	Seed not dormant		
98	Secondary dormancy induced		
99	Secondary dormancy lost		
	<u>Transplanting and recovery (rice only)</u>		
T1	Uprooting of seedlings		
T2	-		
T3	Rooting		
T4	-		
T5	-		

T6	-
T7	Recovery of shoots
T8	-
T9	Resumption of vegetative growth

Notes on the Table

- (1) Stage of seedling inoculation with rust in the greenhouse.
- (2) Only applicable to cereals with a prostrate or semi-prostrate early growth habit.
- (3) Ripeness for binder (ca. 16% water content). Chlorophyll of inflorescence largely lost.
- (4) Ripeness for combine harvester (< 16% water content).
- (5) Optimum harvest time.

ANNEX II

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/058/1