



PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

***Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll**

SUGARBEET COMPONENTS

UPOV Code: BETAA_VUL

Adopted on 21/03/2018

Entry into force on 01/02/2018

I - SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on the general UPOV Document TG/1/2 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to all varieties of *Beta vulgaris* L. ssp. *vulgaris* var. *saccharifera* Alef. (syn. *Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) with the exception of commercial hybrids.

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 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 the 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 whether acceptable plant material has been received or not. However if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Plant material requirements

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on <http://www.cpvo.europa.eu/main/en/home/documents-and-publications/s2-gazette> in the special issue S2 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

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.

The composition of the variety collection in the 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 Office 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 the Examination Office 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 sugarbeet components. 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 Annex 1. 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 characteristic used for grouping are the following:

- None

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 period.

The test design is as follows:

All observations determined by measurement or counting should be made on at least 60 plants or parts of 60 plants divided on and taken from 2 or 3 replications.

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 characters:

In the case of characteristics which show discrete discontinuous states of expression the difference between two varieties is clear if one or more of 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**

A candidate will be considered to be sufficiently uniform if the number of off-types does not exceed the number of plants as indicated in the table below on the basis of a population standard of 2% with an acceptance probability of 95%.

Table of maximum numbers of off-types allowed for uniformity standards

NUMBER OF PLANTS	OFF-TYPES ALLOWED
100 – 131	5
132 – 165	6
166 – 200	7
201 – 236	8
237 – 273	9
274 – 310	10
311 – 348	11
349 – 386	12

c) **Stability**

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

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.

The interim report and final report shall be sent by the Examination Office to the CPVO.

VI - TABLE OF CHARACTERISTICS

CPVO N°	Stage, Method	Characteristics	Examples	Note
1. (+)	10-11 C	Germity: percentage of monogerm seeds		
		monogerm	MS147	1
		partly monogerm/ partly multigerm		2
		multigerm	KW919	3
2. (+)	10-11 C	Seedling: percentage of seedlings with anthocyanin coloration of hypocotyl		
		0 – 19%	MS147	1
		20 – 39%		2
		40 – 59%	MS146	3
		60 – 79%		4
		80 – 100%	MS99202	5
3. (+)	35-39 VG	Leaf: attitude		
		erect		1
		semi-erect		3
		intermediate	MS146	5
		semi-prostrate		7
		prostrate		9
4.	35-39 VG	Leaf blade: green colour		
		light		3
		medium	F99202	5
		dark		7
5.	40-45 VG	Leaf blade: undulation of margin		
		weak	MS111	3
		medium	KWS904	5
		strong	MS144	7

CPVO N°	Stage, Method	Characteristics	Examples	Note		
6.	40-45	Leaf blade: glossiness				
			VG	weak	MS121	3
				medium	KWS903	5
				strong	F99202	7
7.	40-45	Leaf blade: blistering				
			VG	weak	F99203	3
				medium	KWS915	5
				strong		7
8.	40-45	Plant: height				
			MG	short	MS152	3
				medium		5
				tall	POLL132	7
9.	40-45	Leaf blade: length				
			(+) MS	short	MS152	3
				medium	MS151	5
				tall	POLL132	7
10.	40-45	Leaf blade: width				
			(+) MS	narrow	MS152	3
				medium	POLL131	5
				broad	POLL132	7
11.	40-45	Leaf blade: width compared to length				
			(+) MS	narrow	MS111	3
				medium	F99202	5
				broad	Stru2901	7
12.	40-45	Petiole: length				
			(+) MS	short	MS152	3
				medium	KW919	5
				long	POLL132	7

CPVO N°	Stage, Method	Characteristics	Examples	Note
13.	40-45	Petiole: width		
(+)	MS	narrow	F99203	3
		medium	POLL131	5
		broad		7
14.	49	Root: position in soil		
(+)	MS	low	KW905	3
		medium	MS161	5
		high		7
15.	49	Root: length		
(+)	MS	short	MS121	3
		medium	F99203	5
		long	D1019Vh007C	7
16.	49	Root: width		
(+)	MS	narrow	F99203	3
		medium	POLL132	5
		broad	M99202	7
17.	49	Root: width compared to length		
(+)	MS	narrow	F99203	3
		medium	MS147	5
		broad	KW903	7

Legend:

(+) See Explanations hereunder in "explanations and methods"

1) The stages of development denoted by each number are described in Annex 1

The method of assessment is indicated by letters as follows:

MG	Measurement of a group of plants or plant parts
MS	Measurement of a number of individual plants or plant parts
VG	Visual assessment by a single observation of a group of plants or plant parts
C	Special test

ANNEXES TO FOLLOW

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ANNEX II

Technical Questionnaire

ANNEX I

EXPLANATIONS AND METHODS

Ad 1: Germity: percentage of monogerm seeds

3 x 100 seeds from the submitted sample to be grown under ISTA-rules.

Preparation of the seeds for assessment of germity: Prewash seeds for 2 h, dry the seeds for 4 h at max. 25°C, put 100 seeds in pleated paper with 50 folds placed in plastic tray, put 2 seeds per fold, make 3 replications of 100 seeds, add 40 ml of deionised water, put the plastic tray in 20°C for 7 days, assure day length of 12 h.

Assessment by counting the number of monogerm seeds per 100 seeds. The assessment has to be done very carefully in order to avoid typical errors, e.g. assessing not germinated seeds of a multigerm as monogerm or counting twins in monogerm as multigerm.

The attribution of notes for state of expressions is as follows:

Note 1 = monogerm with equal or more than 95% of monogerm seeds

Note 2 = partly monogerm/partly multigerm with less than 95% and more than 15% monogerm seeds

Note 3 = multigerm with equal or less than 15% monogerm seeds

Distinctness is achieved with two notes difference.

Ad 2: Seedling: percentage of seedlings with anthocyanin coloration of hypocotyl

Growing of 3 x 100 seeds from the submitted sample under ISTA rules.

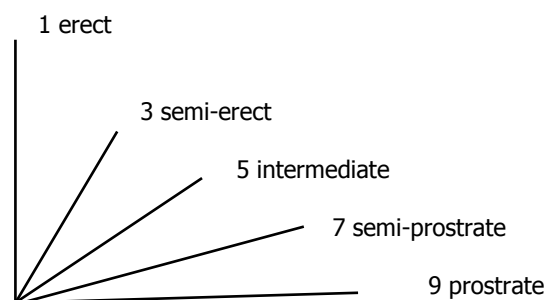
Preparation of the seeds for assessment of anthocyanin coloration of hypocotyl:

Put 100 seeds in pleated paper with 50 folds placed in plastic tray, put 2 seeds per fold, make 3 replications of 100 seeds, add 40 ml of deionised water, put the plastic tray in 20°C for 7 days, assure day length of 12 h.

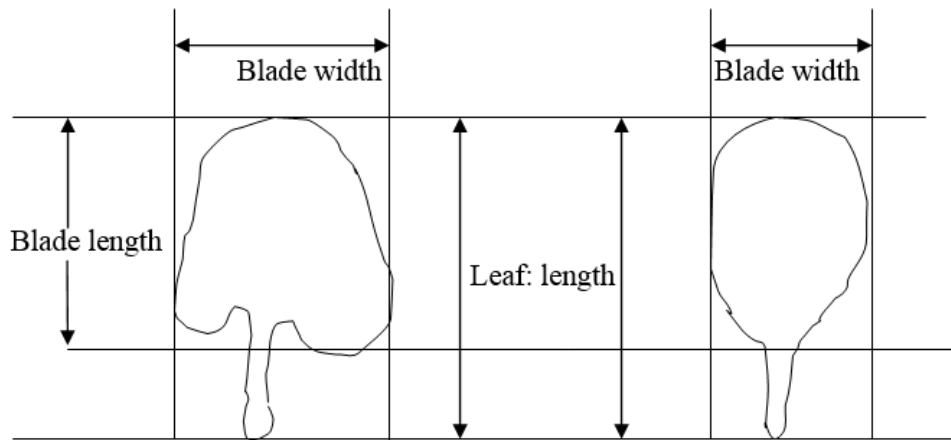
Assessment by counting the number of seedlings with anthocyanin coloration of hypocotyl. Distinctness assessed on the % value, achieved when difference is $\geq 40\%$ between varieties.

Ad 3: Leaf: attitude

The leaf attitude should be measured visually from the angle formed by the average attitude of the petioles and the vertical axis through the root.



Ad 9+10+12: Leaf blade: length and width; Petiole: length
Use leaves from the 2. Row from the bottom of the root.



Ad 11: Leaf blade: width compared to length

Leaf blade: width compared to length has to be calculated as the ratio width: length.

Ad 12: Petiole: length

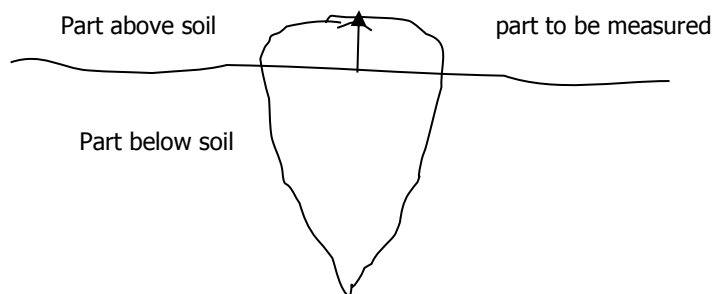
Petiole length has to be calculated as the difference of measured leaf length (including petiole) and leaf blade length.

Ad 13: Petiole: width

The measurement should be taken ca. 3 cm above the base of the petiole.

Ad 14: Root: position in soil

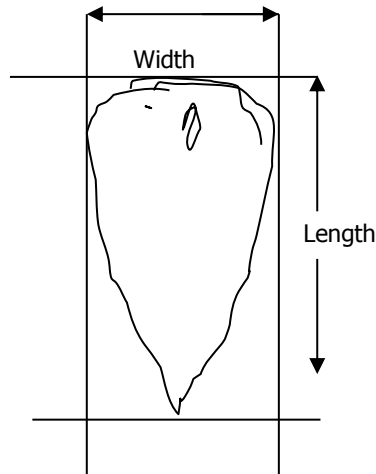
Measurement of the part of the root which exceeds the soil when harvesting single plants.



Ad 15+ 16: Root: length and width

Length: To measure between the root tip at 2 cm diameter and the base of the green petioles.

Width: To measure at the broadest extension of the root, parallel to the top cut.



Ad 17: Root: width compared to length

Root width compared to length has to be calculated as the ratio width: length.

Phenological growth stages according to the BBCH-identification keys of beet

00	Dry seed
10 - 11	Leaf development (youth stage), Seedling
35 - 39	Rosette growth: leaves cover 50% - 90% of ground
40 - 45	Development of harvestable vegetative plant parts – Beet root
49	Beet root has reached harvestable size

Uwe Meier, 1997: Growth Stages of Mono- and Dicotyledonous Plants. Federal Biological Research Center for Agriculture and Forestry (Editor)

ANNEX II

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/X05/1-Partial-Rev