

**UNITED KINGDOM NATIONAL LIST/ PLANT BREEDERS RIGHTS TECHNICAL PROTOCOL FOR THE
OFFICIAL EXAMINATION OF DISTINCTNESS, UNIFORMITY AND STABILITY (DUS)**

SUGAR BEET

(Beta vulgaris L. ssp.vulgaris var.altissima Döll)

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4 NON COMPLIANCE WITH THE PROTOCOL

- 4.1 Where the protocol uses the word “must” for any action then failure to carry out this action will result in non-compliance. Where non-compliance occurs or there are concerns regarding the validity of any data or tests this must be reported to APHA. Where this protocol uses the word “should” for any action this is the method to be followed unless there are clear reasons not to do so which can be justified by the Test Centre as technically sound.

5 RESPONSIBILITY FOR GM RELEASES

- 5.1 GM Release Consent Holders are responsible for GM releases. All parties involved in DUS work operating under a GM Release Consent must adhere to the instructions of the Release Consent Holder where necessary, to comply with the relevant consent conditions. Where DUS protocol non-compliance occurs, this must be reported to the consent holder and the Test Centre who will notify APHA.

6 PROCEDURES FOR GM VARIETIES

- 6.1 Applicants intending to enter GM candidates must consult APHA, well in advance of their application, about specific requirements under GM regulations.
- 6.2 The Test Centre must ensure that no test or trial sites are planted with GM candidates and/or varieties until APHA has given the specific clearances.

7 ASSOCIATED DOCUMENTS

- 7.1 The following documents are associated with this protocol

Reference	Title
Sugar Beet VCU Protocol	United Kingdom National List trials: Protocol and Procedures for Official Examination of Value for Cultivation and Use (VCU) of Sugar Beet.
UPOV TG/1/3	General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonised Descriptions of New Varieties of Plants. 9.4.2002
UPOV TC/33/7	Combined Over-Years Criterion for Distinctness (COYD) and Uniformity (COYU). (Revision of document TC/30/4).
UPOV TGP/8/2	Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability. 16.10.2014
UPOV TGP/9/2	Examining Distinctness. 29.10.2015
UPOV TGP/10/1	Examining Uniformity. 30.10.2008
Commission Directive	Commission Directive of 2003/90/EC as amended setting out implementing measures for the purposes of Article 7 of Council Directive 2002/53/EC (13 June 2002) as regards the characteristics to be covered as a minimum by the examination and the minimum conditions for examining certain varieties of agricultural plant species.
Council Regulation EC No 2100/94	Council Regulation 2100/94/EC of 27 July 1994 on Community Plant Variety Rights.

SECTION B - APPLICATION REQUIREMENTS**1 PURPOSE**

- 1.1 The purpose of this section is to identify the specific requirements for National List and Plant Breeders' Rights applications.

2 SCOPE

- 2.1 These procedures apply to all applications.

3 RESPONSIBILITIES

- 3.1 The applicants are responsible for ensuring that these procedures are complied with.

4 RECEIPT OF APPLICATIONS

- 4.1 The latest date for receipt of applications for acceptance of a variety onto the National List or for Plant Breeders' Rights, which is set administratively by APHA, is 15 January. Applications received after this date may be considered for inclusion in the current year's tests and trials on a case by case basis.

- 4.2 The procedures for the submission of National List and Plant Breeders' Rights applications, technical questionnaires and for payment of administration fees are set out on the APHA web site (<https://www.gov.uk/guidance/national-lists-of-agricultural-and-vegetable-crop>)

- 4.3 Applicants should notify APHA of special DUS characteristics, which may require additional examinations. These claims should, in addition, be noted in the TQ accompanying the application.

5 RECEIPT OF SEED

- 5.1 The latest date for receipt of seed is 1st February and is set administratively by APHA. Seed submissions received after this date will normally be refused. Instructions for the delivery of seed will be made available to applicants by APHA.

6 SEED QUALITY REQUIREMENTS

- 6.1 The seed must meet the standards for the final generation of seed given in the appropriate seed regulations, in respect of germination, analytical purity and content of other seeds and any other impurities.

- 6.2 The seed must not be chemically treated. Seed treatment and pelleting will be carried out by an approved seed pelleting organisation prior to delivery to the Trials Organiser for VCU trials on beet. Currently, Germain's UK, are the approved seed pelleting organisation. The chemicals applied and rates of application will be determined by the Trials Organiser.

- 6.3 Seed should be of the size grade 3.00 to 4.25 mm before pelleting with not more than 25% in the 4.00 to 4.25 mm fraction.

7 SEED QUANTITY

7.1 A new seed submission is required for each year of tests. In years 1, 2 and further years, sufficient seed is submitted for both DUS and VCU National List tests and trials as defined in the current VCU protocol.

7.2 Year 1 Seed

A minimum of 40,000 seeds of the total seed submission is made available for DUS.

7.3 Year 2 and further years' submissions.

A minimum of 40,000 seeds of the total seed submission is made available for DUS

7.3 Shortfall in Seed Quantities

Where sufficient seed is unavailable in the first instance a further stock should be supplied in the following year which will be authenticated against the original submission. An additional charge may be applied.

8 LABELLING REQUIREMENTS, INCLUDING PROVISIONS FOR GM VARIETIES

8.1 Applicants **must** clearly label their seed with the following information;-

- Applicant
- Breeder's Reference number or name
- Type of Seed (DUS Only/Combined submission of DUS and VCU).
- Quantity of seed
- Whether it is a parental line.

8.2 All packages of GM material must be clearly labelled as "GMO" or "Genetically Modified Organism".

SECTION C – GROWING TEST PROCEDURES**1 PURPOSE**

- 1.1 The purpose of this section is to provide details of the procedures used in the growing tests for DUS analysis.

2 SCOPE

- 2.1 These procedures apply to all varieties of Sugar Beet.

3 RESPONSIBILITIES

- 3.1 The Test Centre is responsible for conducting these procedures.
- 3.2 The Test Centre will be responsible for ensuring that no material supplied to them is used for any other purpose than the conduct of these procedures or the release of reference samples for authorised purposes.

4 REFERENCE VARIETIES

- 4.1 The principles governing the selection of reference varieties are set out in Appendix 1.
- 4.2 Seed of reference varieties will be supplied by the BSPB/BBRO.

5 DESIGN OF TESTS

- 5.1 The Test Centre is responsible for selecting a suitable site, which should follow British Sugar contract restrictions i.e. no Sugar Beet or other *Beta* species to be grown in the previous two years.
- 5.2 Field husbandry should follow best local practice for all operations and particularly as regards cultivation, drilling, fertiliser and spray application, use of irrigation, and control of pests and diseases.
- 5.3 DUS Growing Test

Spaced, Adult Plant Test as VCU Procedure plots

Three rows are to be drilled at 0.5m row width, with the same row width between plots. Variation in row spacing of more than 10% between adjoining plots should be notified to the Trials Organiser. All rows of the plot will be harvested for yield and the plot size should be sown to allow a minimum target harvest plot, after trimming, of 10m². A minimum of 3m pathway between plot ends is required to facilitate machine harvesting. There will be four replicates sown.

Candidate and Reference Varieties:

Number of Planting Years:	2 plus an additional year if required
Number of Trial Sites:	1
Number of Replicates:	4
Total Number of Plants Examined/Variety:	80

- 5.4 Protocol and Procedures

All trials should follow the VCU trials protocol and procedures as set down in the documents: Protocol and procedures for examination of Value for Cultivation and Use (see Section A 7.1 above).

5.4.1 Year 1 Trials

The design and randomisation for each trial will be specified by the Test Centre according to the VCU Protocol. Trials are usually four replicate incomplete block designs with no additional treatments. Other designs may be used if appropriate.

Number of Planting Years:	2 plus an additional year if required
Number of Trial Sites:	13 sown Number of Replicates: 4
Number of Plants Examined/Variety/Trial:	600 approx.

5.4.2 Year 2 Trials

As for Year 1 trials.

5.5 Replacement Stock Authentication

Reference Stock replacement seed is requested from the maintainer for each year of trials.

5.6 VCU Seed Authentication

Authentication of VCU seed submissions made in the first year are conducted by examining the trial data.

5.7 Additional DUS Seed Authentication of replacement seed.

The definitive seed stock for a variety is the joint submission for DUS and VCU tests and trials made in the first year.

6 RECORDS AND RECORDING

6.1 All records and plot data should be in a form determined and validated by the Test Centre.

6.2 Characters, recording details and instructions are given in Section D. Any variant and abnormal plants or plants resulting from an adverse reaction to husbandry practice are recorded but excluded from the sample.

6.3 In the first recording year, characters, as indicated in Section D5.1, are measured on all varieties and the data analysed to assess uniformity, where assessed visually, of the candidate variety and to determine the most similar reference varieties. (For details see Section G).

6.4 In the second recording year, characters, as indicated in Section D5.1, are measured on all varieties and the data analysed and, together with those from the first year, used to assess distinctness and uniformity, where assessed visually, of the candidate variety. (For details see Section G).

6.5 If the Test Centre notices unusual or novel characters in candidate varieties a note may be made of these at any time and a photographic record made.

7 COMMUNICATIONS WITH THE APPLICANT

7.1 The Test Centre will notify the applicant or their agent of any DUS problems at the earliest practical opportunity. All such notifications must be copied to APHA.

7.2 If confidentiality considerations allow, the applicant should be informed which variety is similar and be invited to submit any information, which may help to distinguish them.

7.3 If DUS problems arise, applicants will be invited to visit the DUS test by arrangement so that the material can be examined and discussions held with the Test Centre.

- 7.4 After each recording season the results are summarised and reported to the applicant and APHA by the Test Centre.

SECTION D - SUMMARY OF DUS CHARACTERISTICS TO BE ASSESSED, METHOD OF ASSESSMENT AND STANDARDS APPLIED**1 PURPOSE**

1.1 The purpose of this section is to summarise the characteristics to be assessed.

2 SCOPE

2.1 This section summarises characteristics, states of expression, method of observation and standards required for DUS assessment.

3 RESPONSIBILITIES

3.1 The Test Centre is responsible for co-ordinating the procedures in this summary.

4 ORGANISATION

4.1 The minimum duration of tests to assess characteristics should normally be two growing periods. If the variety is not DUS after 2 years of tests, the DUS Test Centre may, with the agreement of the breeder carry out a third year of tests. Shorter periods may be applied for assessment of additional characteristics. Additional growing periods may be approved by the UK National List and Seeds Committee.

5 DUS CHARACTERISTICS TO BE ASSESSED**5.1 Routine Characteristics**

The following table summarises the DUS characteristics to be routinely examined.

G denotes a grouping characteristic.

D denotes a characteristic used in the variety description.

UK Character reference number	Character	Material examined	Number of plants or sample size for assessment	Method of assessment and recording	States of expression	D Method Minimum difference required	U and S Method
100 G D	Monogermity	Submitted seed	Single analysis of submitted bulk	OSTS method based on ISTA rules	% monogerm seeds	1 state	Visual test
101 G D	Ploidy	Submitted seed	Single analysis of submitted bulk, 25 seeds	Flow cytometry	Diploid, triploid, tetraploid, anisoploid	1 state	Visual test
10 D	Petiole width	DUS plot	80 plants per variety from four replicates	Measured	mm	2 @ 5%	Visual test and F3 statistic
11 D	Petiole length	DUS plot	80 plants per variety from four replicates	Measured	mm	2 @ 5%	Visual test and F3 statistic
12 D	Total leaf length	DUS plot	80 plants per variety from four replicates	Measured	mm	2 @ 5%	Visual test and F3 statistic
13 D	Lamina width	DUS plot	80 plants per variety from four replicates	Measured	mm	2 @ 5%	Visual test and F3 statistic
15 D	Lamina shape	DUS plot	80 plants per variety from four replicates	Calculated	mm - length/width	2 @ 5%	Visual test and F3 statistic
16 D	Lamina area	DUS plot	80 plants per variety from four replicates	Calculated	mm - length x width	2 @ 5%	Visual test and F3 statistic
18 D	Total leaf length/lamina length	DUS plot	80 plants per variety from four replicates	Calculated	mm	2 @ 5%	Visual test and F3 statistic
14 D	Lamina length	DUS plot	80 plants per variety from four replicates	Calculated	mm - total length - petiole length	2 @ 5%	Visual test and F3 statistic
17 D	Petiole shape	DUS plot	80 plants per variety from four replicates	Calculated	mm - length/width	2 @ 5%	Visual test and F3 statistic
19 D	Total leaf length/petiole width	DUS plot	80 plants per variety from four replicates	Calculated	mm	2 @ 5%	Visual test and F3 statistic
44 D	Lamina width/petiole width	DUS plot	80 plants per variety from four replicates	Calculated	mm	2 @ 5%	Visual test and F3 statistic
74D	Leaf Blistering	VCU trials	Whole plot	Visual plot score on 4 replicates	1 to 9 smooth to very blistered	2 @ 5%	Visual test
73D	Foliage colour	VCU trials	Whole plot	Visual plot score on 4 replicates	1 to 9 light green to dark green	2 @ 5%	Visual test
72D	Leaf waving	VCU Trial	Whole plot	Visual plot score on 4 replicates	1 to 9 smooth to very wavy	2 @ 5%	Visual test
71D	Foliage Habit	VCU Trial	Whole plot	Visual plot score on 4 replicates	1 to 9 prostrate to erect	2 @ 5%	Visual test

UK Character reference number.	Character	Sample source: material examined	Sample number or size for assessment & recording	Method of assessment & recording	States of Expression	D Method Minimum difference required	U and S Method
8 D	Top size	VCU Trial	Whole plot	Visual plot score on 4 replicates	1 to 9 small to large	2 @ 5%	Visual test
45 D	Crown height	VCU Trial	40 plants per variety from four replicates	Visual plant scores on 40 plants	1 to 9 low to high	2 @ 5%	Visual test
55 D	Amino N	VCU trials	Whole plot	Measured	meq. per 100g sugar	2 @ 5%	F3 statistic
5 D	Amino N	VCU trials	Whole plot	Measured	meq. per 100g beet	2 @ 5%	F3 statistic
90 D	Amino N	VCU trials	Whole plot	Measured	mg. per 100g sugar	2 @ 5%	F3 statistic
4 D	Potassium	VCU trials	Whole plot	Measured	meq. per 100g beet	2 @ 5%	F3 statistic
22 D	Potassium	VCU trials	Whole plot	Measured	meq. per 100g sugar	2 @ 5%	F3 statistic
3 D	Sodium	VCU trials	Whole plot	Measured	meq. per 100g beet	2 @ 5%	F3 statistic
33 D	Sodium	VCU trials	Whole plot	Measured	meq. per 100g sugar	2 @ 5%	F3 statistic
6 D	Root yield	VCU trials	Whole plot	Measured	Tonnes/ha	2 @ 5%	F3 statistic
1 D	Sugar percentage	VCU trials	Whole plot	Measured	%	2 @ 5%	F3 statistic
66 D	Sugar yield	VCU trials	Whole plot	Calculated	Tonnes/ha	2 @ 5%	F3 statistic
2 D	Total impurities	VCU trials	Whole plot	Calculated	meq. per 100g sugar	2 @ 5%	F3 statistic

5.2 Additional Characters (See also Section G 6.1)

UK Character reference number.	Character	Material examined	Number of plants or sample size for assessment	Method of assessment & recording – see Fodder Beet method TG/150/3	States of Expression	D Method and Minimum difference required	U Method
93	Hypocotyl axis: colouring	Glasshouse test	Whole plot	Visual Plot score on 2 replicates	White, green, yellow, orange, pink, red, dark red or combinations of the above	2 @ 5%	Visual test and F3 statistic

5.3 New Additional DUS Characteristics

Applicants can suggest new additional characters on the Technical Questionnaire for testing DUS or after notification by the DUS Test Centre of distinctness problems. For procedures see Section F.

SECTION E - REFERENCE SEED STOCK MAINTENANCE AND VCU SEED STOCK AUTHENTICATION PROCEDURES**1 PURPOSE**

- 1.1. This section sets out the procedures for reference seed stock maintenance and VCU seed stock authentication.

2 SCOPE

- 2.1 These procedures apply to all reference collection varieties and VCU seed submissions where the VCU seed has been taken from the same bulk as the seed used for the DUS test.

3 RESPONSIBILITIES

- 3.1 The Test Centre is responsible for conducting these procedures.

4 PROCEDURES FOR REFERENCE SEED STOCK MAINTENANCE

- 4.1 The seed sample submitted with the successful or pending application is considered to be the definitive stock of the variety.
- 4.2 A new submission of reference seed is required for each year of tests. Sufficient seed is submitted for both DUS and VCU National List tests and trials.
- 4.3 Data obtained during the course of the tests and trials will be used to authenticate reference seed submissions from different years.

5 PROCEDURES FOR VCU SEED STOCK AUTHENTICATION

- 5.1 A new seed submission is required for each year of tests. In years 1, 2 and further years, sufficient seed is submitted for both DUS and VCU National List tests and trials.
- 5.2 Data obtained during the course of the trials will be used to authenticate the VCU seed submissions from different years.

SECTION F- PROCEDURES FOR ASSESSMENT OF NEW ADDITIONAL DUS CHARACTERS**1 PURPOSE**

- 1.1 This Section sets out the procedures for assessment of new additional DUS characters for varieties of Sugar Beet entered for National List trials and PBR.

2 SCOPE

- 2.1 These procedures apply to applications where new additional DUS characteristics, which have not been approved by the NLSC, are requested for use for determinations of DUS.

3 RESPONSIBILITIES

- 3.1 The Test Centre is responsible for liaising with the applicant to produce a proposed procedure for the conduct of new tests. This procedure must ensure that Distinctness, Uniformity and Stability will be assessed.
- 3.2 All new additional characteristics must be authorised by the National List and Seeds Committee.

4 REFERENCE VARIETIES

- 4.1 The reference varieties will include only those varieties from which the candidate variety is not distinct, as well as other appropriate varieties for control purposes.
- 4.2 Seed of reference varieties will be supplied by the Test Centre.

5 PROCEDURES

- 5.1 Details of the proposed special test or assessments will be submitted to the NLSC to consider the feasibility of setting up a test acceptable to the UK Authorities. The applicant will be advised by APHA of arrangements and costs.
- 5.2 The NLSC will consider the results of the commissioned test or trial when reaching its recommendation on the granting of Plant Breeders' Rights and/or National Listing.
- 5.3 Where the test for a character is approved by the NLSC it should be subsequently listed in Section D5.1 or 5.2 as appropriate.

SECTION G - PROCEDURES FOR DUS DECISIONS**1 PURPOSE**

1.1 This section sets out the procedures for assessing DUS decisions on varieties of Sugar Beet.

2 SCOPE

2.1 These procedures apply to all varieties of Sugar Beet entered for National List and Plant Breeders' Rights tests and those being tested for Foreign Authorities.

3 RESPONSIBILITIES

3.1 The Test Centre is responsible for applying the criteria for DUS, set out in this procedure.

3.2 The Test Centre is responsible for producing the DUS reports in accordance with these procedures and for ensuring that they are in accordance with the UPOV Guidelines.

4 REFERENCE VARIETIES

4.1 Appendix 1 sets out which varieties are considered as reference varieties for these procedures.

5 DISTINCTNESS

5.1 In accordance with associated document UPOV TG1/3 varieties can be considered distinct where they have a different expression in a grouping character e.g. ploidy, germity (monogerm or multigerm) and utilisation type.

5.2 A variety is considered distinct, after two years, if in each year of test the t-values between the candidate variety and other varieties is significant at the 5% level ($P=0.05$) in the same direction.

5.3 A variety is considered distinct, after three years, if for two years of test the t-values between the candidate variety and other varieties is significant at the 5% level ($P=0.05$) in the same direction without there being a reversal of direction in the third year.

5.4 Where varieties are grown in close proximity under the same conditions, and a direct comparison can be made, observations can be made on differences not revealed by single spaced plant trials and further observations on single spaced plant trials or special tests can be undertaken.

6 UNIFORMITY

6.1 Uniformity is only assessed by a visual test on characters indicated in section D. For the hypocotyl test using the Fodder Beet method varieties showing two colours should not be regarded as lacking uniformity.

6.2 Any outlier plants (off-types) are identified by the analysis and decisions taken by the Test Centre on whether they should be excluded or not from the calculation of variety means and standard deviations. Off-type plants in the field are identified by visual assessment and are marked for a decision on omission for recording depending upon incidence across replicates.

7 STABILITY

7.1 A variety is considered sufficiently stable when there is no evidence to indicate that it lacks stability indicated by a significantly high F3 or fails to conform to the essential characteristics of its description in different submissions or in different tests.

8 DUS REPORT AND VARIETY DESCRIPTION

- 8.1 Upon completion of the DUS examination the DUS Summary report will be submitted to APHA by the specified date. This report will specify all non-routine characteristics for establishing distinctness.
- 8.2 The final DUS report, including the full variety description, will be submitted to APHA by the specified date. The characteristics to be used in the description are identified in Section D.

APPENDIX 1**REFERENCE COLLECTION VARIETIES****1 NATIONAL LISTING**

- 1.1 The DUS reference collection, comprises the following at the time when the application for the candidate is made:
- 1.1.1 All other candidate varieties already in DUS test in the UK, or entering testing at the same time as the candidate, including those being tested for other Member States.
 - 1.1.2 Varieties on the UK National List grown commercially in the UK plus those entered for export
 - 1.1.3 Varieties on the EC Common Catalogue whose seed is known to be certified or marketed in the UK.
 - 1.1.4 Varieties nominated by the authorities concerned where the tests are done for other Member States
 - 1.1.5 Any varieties nominated by the applicant as being comparable i.e. known to be similar.
 - 1.1.6 Any other varieties considered to be comparable i.e. known to be similar by the appropriate DUS Test Centre.

2 PLANT BREEDERS RIGHTS

- 2.1 The DUS reference collection, for PBR purposes, for any given category of plant variety comprises the following at the time when the application for the candidate is made:
- 2.1.1 All other candidate varieties already in DUS test in the UK, or entering DUS testing at the same time as the candidate, including those being tested for other Member States or the Community Plant Variety office (CPVO).
 - 2.1.2 Varieties protected in the UK, EC or in a UPOV Member State, which are known to be similar to the candidate variety.
 - 2.1.3 Other available comparable varieties in common knowledge.