



Animal &  
Plant Health  
Agency

# Great Britain and Northern Ireland Variety Lists/Plant Breeders' Rights Technical Protocol for Official Examination of Distinctness, Uniformity and Stability (DUS)

## Sugar Beet

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

March 2024

## Summary of changes made

- Changed year of document
- Changed United Kingdom to Great Britain and Northern Ireland
- Standard wording inserted.
- Removal of outstanding EU references, including within the table of characteristics
- Appendix 1 updated
- Approved additional characteristics added to section D5.3. (Hypocotyl and cotyledon seedling test and ALS test)
- Updates to table of characteristics (section 5.2) to make it clearer the number of trials, replicates and plants observed for each characteristic
- Updates to seed quality requirements
- Appendices 2 and 3 (Special Tests)

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# Section A – general information

## 1. Purpose

1.1 This Protocol sets out the procedures for conducting tests and assessments in relation to official examinations of DUS, maintenance of reference stocks and verification of VCU submissions of varieties of Sugar Beet entered for Variety Listing (VL) Trials and/or Plant Breeders' Rights (PBR).

## 2. Scope

2.1 These procedures apply to all varieties of Sugar Beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll). Special procedures and responsibilities for Genetically Modified (GM) varieties are set out in Sections A5 and A6.

2.2 Except where specified in this protocol or authorised by the Plant Variety Rights Office for the UK, Animal and Plant Health Agency (APHA); only Variety Lists candidates, Plant Breeders' Rights candidates, candidates for Foreign Authorities and the reference varieties may be incorporated in the DUS tests.

## 3. Responsibilities

3.1 The growing tests and assessments in this protocol are carried out under the responsibility of the Secretary of State for Environment, Food and Rural Affairs, Scottish ministers, Welsh ministers and the Minister for Agriculture, Environment and Rural Affairs in Northern Ireland (the National Authorities).

3.2 They are supervised, on behalf of the National Authorities, by officials of the Testing Authorities: APHA; the Scottish Government (SG); the Department of Agriculture, Environment and Rural Affairs (DAERA); and Welsh Government (WG).

3.3 This protocol is authorised by the Plant Variety and Seeds Committee (PVSC). It cannot be amended without its approval. Requests and suggestions for amendment of the protocol should be put in writing to APHA Plant Varieties and Seeds, either directly or via the Test Centre.

3.4 The procedures are administered by:

Plant Variety Rights Office for the UK  
Animal and Plant Health Agency  
Eastbrook  
Shaftsbury Avenue  
Cambridge

CB2 8DR

Email: [pvs.helpdesk@apha.gov.uk](mailto:pvs.helpdesk@apha.gov.uk)

### 3.5 Test Centre

The DUS assessments in this protocol are co-ordinated and carried out by the Test Centre:

NIAB  
Barn 1 Park Farm  
Villa Road  
Impington  
Cambridge  
CB24 9NZ

Telephone: 01223 342200

Field Trials are organised by:

British Society of Plant Breeders Ltd (BSPB)  
BSPB House  
114 Lancaster Way Business Park  
Ely  
Cambridgeshire  
CB6 3NX

Telephone: 01353 653846

Glasshouse Trials are co-ordinated and carried out by:

NIAB  
Barn 1 Park Farm  
Villa Road  
Impington  
Cambridge  
CB24 9NZ

Telephone: 01223 342200

3.6 The Test Centre is responsible for providing the appropriate facilities.

## 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 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 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 or varieties, or both until APHA has given the specific clearances.

## 7. Associated documents

7.1 These reference documents are associated with this protocol:

- Sugar Beet VCU Protocol – United Kingdom Variety 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 TGP/8/4 – Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability. 01.11.2019
- UPOV TGP/9/2 – Examining Distinctness. 29.10.2015
- UPOV TGP/10/2 – Examining Uniformity. 01.11.2019
- GB and NI Variety Lists – The Seeds (National Lists of Varieties) Regulations 2001 (as amended) and The Seeds (Variety Lists) Regulations (Northern Ireland) 2020
- Plant Varieties Act 1997 – Plant Breeders' Rights Regulations 1998 and Plant Varieties Act 1997
- Plant Breeders' Rights 2019: The Plant Breeders' Rights (Amendment etc.) (EU Exit) Regulations 2019 as amended by The Animal Health, Invasive Alien Species, Plant Breeders' Rights and Seeds (Amendment etc.) (EU Exit) Regulations 2019 and The Plant Breeders' Rights (Amendment) (EU Exit) Regulations 2020



## Section B – application requirements

### 1. Purpose

1.1 The purpose of this section is to identify the specific requirements for Variety Listing and/or Plant Breeders' Rights applications, as appropriate.

### 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 Variety Listing and/or for Plant Breeders' Rights](#) is stated on the GOV.UK website.

4.2 The procedures for the submission of Variety Listing and/or Plant Breeders' Rights applications, Technical Questionnaires (TQs) and for payment of administration fees can be obtained from APHA at the address shown in Section A or on the [GOV UK website](#).

4.3 Applicants should note in the TQ, submitted in the application, any additional characteristics which may require examinations that are listed in the DUS characteristics section D, 5.3 or 5.4 (an additional fee may be required).

### 5. Receipt of seed

5.1 The latest date for receipt of seed is stated in the Seed Gazette. In the absence of exceptional circumstances, seed submissions received after this date will be refused. Read [instructions for the delivery of seed](#).

### 6. Seed quality requirements

6.1 The seed must satisfy the certification requirements for certified seed of the first generation as laid down in the seed marketing legislation of the Devolved Administrations.

6.2 Pelleted seed is supplied by the applicants according to instructions in the VCU protocol and procedures. Pelleting material must be a standard commercial pellet of inert material, dressed with an appropriate seed dressing, with no bio-stimulant additives as stipulated in the VCU protocol and procedures.

## 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 Variety Lists tests and trials as defined in the current VCU protocol.

7.2 Total seed submission for each variety is determined by the VCU trial co-ordinator.

## 8. Labelling requirements, including provisions for GM varieties

8.1 Applicants must clearly label their seed, inside and outside the bag, with the following information:

- Applicant
- AFP number (if known)
- Breeder's reference number or name
- Quantity of seed
- Seed treatments applied
- Pellet weight

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

## Section C – growing pest 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 it is used for any other purpose than the conduct of these procedures or the release of reference samples for authorised purposes (See Section E7).

### 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 Trials Organiser for Variety Listing and the DUS Test Centre for PBR (if applicable).

### 5. Design of tests

5.1 All field trials are conducted according to the VCU trials protocol and procedures for sugar beet as set down in the documents: Protocol and procedures for examination of Value for Cultivation and Use (see Section A 7.1 above).

5.2 Crop husbandry should follow 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 The minimum duration of tests should normally be two independent growing cycles. Additional growing cycles may be approved by the National List and Seeds Committee (NLSC).

5.4 Field Trials

Three rows are to be drilled at 0.5 m 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 10 m<sup>2</sup>. A minimum of 3 m pathway between plot ends is required to facilitate machine harvesting. There will be four replicates sown.

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

<b>Candidate and Reference Varieties</b>	
Number of Planting Years	2 plus an additional year if required
Number of Trial Sites per Year	6 sown, 4 harvested
Number of Replicates per Trial	4

### 5.5 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).

### 5.6 Replacement Stock Authentication

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

### 5.7 VCU Seed Authentication

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

### 5.8 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 analysis.

6.3 In each recording cycle, characters, as indicated in Section D 5.2, 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 cycle, characters, as indicated in Section D 5.2, are measured on all varieties and the data analysed and, together with those from the first year, used to determine overall distinctness, uniformity and stability 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 must 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 the agent of any DUS problems at the earliest practical opportunity, as they arise during the test. All such notifications must be copied to APHA.

7.2 In the case of distinctness problems, if confidentiality considerations allow, the applicant should be informed which variety is not distinct 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 (if appropriate) 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 of characteristics.

## 4. Organisation

4.1 The minimum duration of tests to assess characteristics is normally two independent growing cycles. Shorter durations may be applied for assessment of additional characteristics. Proposed changes to the number of growing cycles must be approved by the NLSC.

## 5. DUS Characteristics to be assessed

### 5.1 Routine Characteristics

Table in section 5.2 summarises the DUS characteristics to be routinely examined.

Note:

- \* – characteristic which must be examined according to UPOV Guidelines
- G – a grouping characteristic

Type of observation of characteristics:

- MG – single measurement of a group of plants or parts of plants
- MS – measurement of a number of individual plants or parts of plants
- VG – visual assessment by a single observation of a group of plants or parts of plants
- VS – visual assessment by observation of individual plants or parts of plants

## 5.2 Sugar Beet characteristics routinely recorded in DUS tests

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	<b>Monogermity</b>	Submitted seed	MG – Single analysis of submitted bulk	OSTS method based on ISTA rules	% monogerm seeds	1 state	Visual test
101 G	<b>Ploidy</b>	Submitted seed	MG – Single analysis of submitted bulk, 25 seeds	Flow cytometry	Diploid, triploid, tetraploid, anisoploid	1 state	Visual test
10	<b>Petiole width</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Measured	mm	2 @ 5%	Visual test and F3 statistic
11	<b>Petiole length</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Measured	mm	2 @ 5%	Visual test and F3 statistic
12	<b>Total leaf length</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Measured	mm	2 @ 5%	Visual test and F3 statistic
13	<b>Lamina width</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Measured	mm	2 @ 5%	Visual test and F3 statistic
15	<b>Lamina shape</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Calculated	mm - length/width	2 @ 5%	Visual test and F3 statistic
16	<b>Lamina area</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Calculated	mm - length x width	2 @ 5%	Visual test and F3 statistic
18	<b>Total leaf length/lamina length</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Calculated	mm	2 @ 5%	Visual test and F3 statistic
14	<b>Lamina length</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Calculated	mm - total length - petiole length	2 @ 5%	Visual test and F3 statistic
17	<b>Petiole shape</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Calculated	mm - length/width	2 @ 5%	Visual test and F3 statistic
19	<b>Total leaf length/petiole width</b>	VCU trial (1 trial x 4 replicates)	MS – 80 plants (20 plants per variety per replicate)	Calculated	mm	2 @ 5%	Visual test and F3 statistic

44	<b>Lamina width/petiole width</b>	VCU trial (1 trial x 4 replicates)	MS – 8 0 plants (20 plants per variety per replicate)	Calculated	mm	2 @ 5%	Visual test and F3 statistic
74	<b>Leaf blistering</b>	VCU trials (3 trials x 4 replicates)	VG – Whole plot	Visual	1 to 9 smooth to very blistered	2 @ 5%	Visual test and F3 statistic
73	<b>Foliage colour</b>	VCU trials (3 trials x 4 replicates)	VG – Whole plot	Visual p	1 to 9 light green to dark green	2 @ 5%	Visual test and F3 statistic



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
72	<b>Leaf waving</b>	VCU trials (3 trials x 4 replicates)	VG – Whole plot	Visual	1 to 9 smooth to very wavy	2 @ 5%	Visual test and F3 statistic
71	<b>Foliage Habit</b>	VCU trials (3 trials x 4 replicates)	VG – Whole plot	Visual	1 to 9 prostrate to erect	2 @ 5%	Visual test and F3 statistic
8	<b>Top size</b>	VCU trials (3 trials x 4 replicates)	VG – Whole plot	Visual	1 to 9 small to large	2 @ 5%	Visual test and F3 statistic
45	<b>Crown height</b>	VCU trials (3 trials x 4 replicates)	VS – 40 plants (10 plants per variety per replicate)	Visual	1 to 9 low to high	2 @ 5%	Visual test and F3 statistic
55	<b>Amino N</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	meq. per 100g sugar	2 @ 5%	F3 statistic
5	<b>Amino N</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	meq. per 100g beet	2 @ 5%	F3 statistic
90	<b>Amino N</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	mg. per 100g sugar	2 @ 5%	F3 statistic
4	<b>Potassium</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	meq. per 100g beet	2 @ 5%	F3 statistic
22	<b>Potassium</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	meq. per 100g sugar	2 @ 5%	F3 statistic
3	<b>Sodium</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	meq. per 100g beet	2 @ 5%	F3 statistic
33	<b>Sodium</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	meq. per 100g sugar	2 @ 5%	F3 statistic
6	<b>Root yield</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	Tonnes/ha	2 @ 5%	F3 statistic
1	<b>Sugar percentage</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	%	2 @ 5%	F3 statistic
66	<b>Sugar yield</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	Tonnes/ha	2 @ 5%	F3 statistic
2	<b>Total impurities</b>	VCU trials (all harvested trials)	MG – Whole plot	Calculated	meq. per 100g sugar	2 @ 5%	F3 statistic

## 5.3 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
102	<b>Hypocotyl axis: colouring</b>	Glasshouse test on seedlings	VS – 50 plants x 2 replicates per year of seed	Visual	White, green, yellow, orange, pink, red, dark red or combinations of the above	2 @ 5%	Visual test and F3 statistic
103	<b>Hypocotyl axis: presence/absence of colouration</b>	Glasshouse test on seedlings	VS – 50 plants x 2 replicates per year of seed	Visual	Present, absent	2 @ 5%	Visual test and F3 statistic
104	<b>Herbicide tolerance: ALS inhibitor</b>	Glasshouse test on seedlings	VS – 50 plants x 3 replicates per year of seed	Visual	Susceptible, tolerant	2 @ 5%	Visual test and F3 statistic
76	<b>Cotyledon: width</b>	Glasshouse test on seedlings	MS – 20 plants x 2 replicates per year of seed	Measured by Image analysis	mm	2 @ 5%	Visual test and F3 statistic
75	<b>Cotyledon: length</b>	Glasshouse test on seedlings	MS – 20 plants x 2 replicates per year of seed	Measured by Image analysis	mm	2 @ 5%	Visual test and F3 statistic
81	<b>Cotyledon: wide point</b>	Glasshouse test on seedlings	MS – 20 plants x 2 replicates per year of seed	Measured by Image analysis	mm	2 @ 5%	Visual test and F3 statistic
80	<b>Cotyledon: width/ cotyledon length ratio</b>	Glasshouse test on seedlings	MS – 20 plants x 2 replicates per year of seed	Calculated	mm	2 @ 5%	Visual test and F3 statistic

## 5.4 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 (if applicable).

## 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 Variety Lists 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 Variety Lists 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.

## 6. Release of reference samples for authorised purposes

6.1 A maximum of 200g of seed of reference samples can be supplied by the Test Centre, on request, to UK and UPOV DUS Testing Authorities and UK and OECD Seed Certification

Agencies. The recipient will be notified in writing that this material, or any material derived from it, must not be supplied to a Third party or used for any other purpose than as a reference for official DUS testing or seed certification.

6.2 Provision of reference samples, other than in 6.1, to any other parties must be authorised by APHA.

# 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 Variety Listing and/or PBR trials.

## 2. Scope

2.1 These procedures apply to applications where additional DUS characteristics, which have not been previously approved by the NLSC, are requested for use in the examination 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 (DUS) requirements will be met.

3.2 All new additional characteristics must be authorised by the NLSC in consultation with the PVSC.

## 4. Reference varieties

4.1 The reference varieties must 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.

5.2 The NLSC may commission a test or trial to further investigate a proposal. The applicant will be advised by APHA of arrangements and costs.

5.3 Where the test for a character is approved by the NLSC it should be subsequently listed in Section D 5.2 or 5.3 as appropriate.

## Section G – procedures for DUS decisions

### 1. Purpose

1.1 This section sets out the standards used to assess DUS of varieties of Sugar Beet.

### 2. Scope

2.1 These procedures apply to all varieties of Sugar Beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) entered for Variety Listing and/or 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 for example, 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 varieties showing two or more 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 and will be discussed at the relevant DUS Test Centre Meeting. This report will specify all non-routine characteristics for establishing distinctness.

8.2 The final DUS report, including the full variety description for positive reports, will be submitted to APHA. The characteristics to be used in the description are identified in Section D.

# Appendix 1 – reference collection varieties

## 1. National listing and plant breeders' rights

1.1 The DUS reference collection, for any given category of plant variety comprises the following at the time when the application for the candidate is made:

1.2 All other candidate varieties already in DUS test in the UK or entering testing at the same time as the candidate.

1.3 All varieties on the GB and NI National Lists including any entered for export only to another member state.

1.4 All varieties with UK PBR.

1.5 All varieties on the OECD variety list that are listed by countries with comparable climatic conditions to UK.

1.6 Any varieties nominated by the applicant as being comparable, meaning that it is known to be similar.

1.7 Any other varieties considered to be comparable, meaning that it is known to be similar by the appropriate Test Centre or DUS Centre Group.



# Appendix 2 – procedures for the herbicide tolerance to ALS inhibitor special test

## 1. Purpose

1.1 The purpose of this section is to provide details of the procedures used in the ALS (Acetolactate synthase) inhibitor tolerance special test used for establishing distinctness in Sugar Beet DUS testing.

## 2. Scope

2.1 These procedures apply to all varieties of Sugar Beet where the ALS special test has been requested.

### Design of tests

3.1 The ALS special test is offered to applicants of varieties which are not yet clearly distinct at the end of 2 years of test.

3.2 The test is carried out on the requested candidate varieties and the varieties found not yet clearly distinct from the candidate.

3.3 The test is carried out in the glasshouse and the varieties sown in multi-cell trays to allow growth of the plants to 4 true leaf growth stage. Glasshouse husbandry should follow best practice for all operations and particularly as regards potting, sowing, lighting, irrigation, and control of pests and diseases. The seed submissions from years 1, 2 and 3 of test are sown as follows:

- Number of replicates – 3 for each annual submission of seed
- Number of plants sown per variety – 50 plants per replicate
- Time of sowing – Mid-February

3.4 Control plots of known ALS inhibitor tolerant and susceptible varieties  $\pm$  herbicide spray application are included within the trial.

3.5 ALS inhibitor herbicide is applied to the plants at the 4 true-leaf stage (usually approx. 4 weeks after sowing depending on growth and development of the plants), following the usage and safety instruction on the label and at the rate specified by the manufacturer.

## **Records and recording**

- 4.1 All records and data should be in a form determined and validated by the Test Centre.
- 4.2 Scoring of symptoms (tolerance and susceptibility) is from 14 to 28 days following herbicide application.
- 4.3 Recording details are summarised in section D 5.3.

## **Communication with the applicant**

- 5.1 After the test and analysis has been completed a summary of the results will be reported to the applicant and APHA by the Test Centre.

# Appendix 3 – procedures for the hypocotyl and cotyledon special tests

## 1. Purpose

1.1 The purpose of this section is to provide details of the procedures used in the hypocotyl and cotyledon special tests used for establishing distinctness in Sugar Beet DUS testing.

## 2. Scope

2.1 These procedures apply to all varieties of Sugar Beet where the hypocotyl and cotyledon special tests have been requested.

### Design of tests

3.1 The hypocotyl and cotyledon special tests for distinctness are declared on the TQ at the time of application. The tests are carried out on all candidate varieties which are not yet clearly distinct at the end of 2 years of test, if a request to withdraw them from these tests has not been received by the closing date for receipt of trial seed at the beginning of the third year (usually 1 February).

3.2 The tests are carried out on the candidate varieties, which have not been withdrawn, and the varieties found not yet clearly distinct from them.

3.3 The tests are carried out in the glasshouse and the varieties sown in multi-cell trays to allow growth of the plants to full cotyledon expansion (adequate growth is usually present after 2 weeks for hypocotyl colouration and 3 to 4 weeks for full cotyledon expansion). Glasshouse husbandry should follow best practice for all operations and particularly as regards potting, sowing, lighting, irrigation, and control of pests and diseases. The seed submissions from years 1 and 2 of test, and year 3 where relevant, are sown in a randomised design as follows:

- Number of replicates – 2 for each annual submission of seed
- Number of plants sown per variety – 50 plants per replicate
- Time of sowing – mid-February

## **Records and recording**

- 4.1 All records and data should be in a form determined and validated by the Test Centre.
- 4.2 Hypocotyl colouration is assessed visually for each seedling plant when they are approximately 5cm tall, usually around 2 weeks after sowing, depending on growth and development of the plants. Varieties showing two or more colours amongst the plants observed in the test should not be regarded as lacking uniformity or stability, provided that the colours observed are consistent across the different seed submissions.
- 4.3 Cotyledon characteristics are measured when the cotyledons are fully expanded after 3 to 4 weeks of growth. One cotyledon per plant is detached and measured from each of 20 plants.
- 4.4 Recording details are summarised in section D 5.3.

## **Communication with the applicant**

- 5.1 After the tests and analysis have been completed, a summary of the results will be reported to the applicant and APHA by the Test Centre.



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