



Animal &  
Plant Health  
Agency

# **United Kingdom National List Technical Protocol for Official Examination of Distinctness, Uniformity and Stability (DUS)**

## **Barley**

### ***Hordeum vulgare* L. sensu lato**

**May 2020**

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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 winter and spring Barley entered for National List (NL) Trials and Plant Breeders' Rights (PBR).

1.2 This Protocol version, dated May 2020, applies to all new candidates entered into test from 1st August 2019. Candidates already in test prior to this date will complete testing under previous UK Protocol version, dated 12/09/2012.

## 2 Scope

2.1 These procedures apply to all varieties of Barley. 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 Animal and Plant Health Agency (APHA) Varieties and Seeds, only National List 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, the Welsh Ministers and the Minister for Agriculture and Rural Development in Northern Ireland (the National Authorities).

3.2 They are supervised, on behalf of the National Authorities, by officials of the Testing Authorities, that is, APHA, Scottish Government Agriculture and Rural Development Division (SGARD), the Department of Agriculture and Rural Development for Northern Ireland (DARDNI) and the Welsh Government (WG).

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

3.4 The procedures are administered by:

Plant Varieties and Seeds  
The Animal and Plant Health Agency  
Eastbrook  
Shaftesbury Road  
Cambridge  
CB2 8DR

Tel No: 0208 026 5993

Fax No: 0208 415 2504

### 3.5 Test Centre

The DUS growing tests and assessments in this protocol are co-ordinated and carried out by:

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

Tel No: 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 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

17.1 The following documents are associated with this protocol

Reference	Title
Barley VCU Protocol	United Kingdom National List Trials: Protocol for Official Examination of Value for Cultivation and Use (VCU) of Cereals (wheat, barley, oats, triticale, rye and spelt wheat)
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 (19.04.2002).
CPVO–TP 019/5	Protocol for Distinctness, Uniformity and Stability Tests of Barley, EU Community Plant Variety Office (19.03.2019).
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).
UPOV TG19/11	Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, Barley (20.09.2018).
Commission Directive	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	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 31st August for Winter Barley and 30th November for Spring Barley. 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 (TQs) and for payment of administration fees can be obtained from APHA PVS at the address shown in Section A or on the website GOV.UK.

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.

4.4 In the case of hybrid varieties the TQ must include details of the nature of the hybrid and all parental lines.

## 5 Receipt of Seed

5.1 The latest date for receipt of seed is 15th September for Winter Barley and 15th January for Spring Barley 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 satisfy the quality requirements for Basic Seed as laid down in Schedule 2 Part 2 of the Seed Marketing Regulations (England) 2011 and equivalent regulations made by the Devolved Administrations. 6.2 The seed must not be chemically treated. Seed treatment, if required, will be undertaken by the Test Centre. The chemicals applied and rates of application will be determined by the Test Centre.

## 7 Seed Quantity

### 7.1 Year 1

Type	Description	Weight
Conventional Type		2 kg* (can be submitted as 1.5 kg bulk seed and 0.5 kg selected seed) with 1000 seed weight given
Single Cross Hybrids	Hybrid Male sterile (female parent) Pollinator (male parent)	2 kg of each line* (can be submitted as 1.5 kg bulk seed and 0.5 kg selected seed) with 1000 seed weight given
Parental lines already under test or already on the UK National List or with UK PBR need not be supplied		
Other Types	Contact APHA	

\*There is a separate submission of seed for VCU trials in Year 1.

### 7.2 Year 2 and Further Year Submissions

None for DUS

A sample of 200g of seed will be withdrawn from VCU submissions in Year 2 and any further years to authenticate the submission (see Section E5). Applicants should refer to Trial Procedures for Official Examination of Value for Cultivation and Use (VCU) for Cereals for seed requirements and Section E4 dealing with replacement seed of a variety.

### 7.3 Shortfall in Seed Quantities

Where insufficient seed is available 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.

## 7.4 Hybrid Barley

In the case of Hybrid Barley where insufficient seed stocks of parent lines are available, a minimum of 500g of each line should be supplied in the first instance. Further stocks should be supplied in the following year and these will be authenticated against the original submission for which an additional charge may be applied. Where components of hybrids are on the UK National List, or have UK PBR, seed need not be supplied unless specifically requested.

## 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
- Breeder's Reference number or name
- Type of Seed (Bulk or Selected, DUS only)
- Quantity of seed and thousand seed weight
- In the case of hybrids, 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 Barley.

## 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. (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 Test Centre.

## 5 Design of Tests

5.1 The Test Centre is responsible for selecting a suitable site which should be on ground that has not normally had a Barley crop in the previous three years but may be less where the risk of contamination is negligible.

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 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 From information given in the TQ the candidate variety may be grown in field grown plots and compared with varieties which are in the same classification for the following grouping characters; seasonal type; presence or absence of sterile spikelets in the ear; presence or absence of lower leaf sheath hairs; number of rows in the ear; rachilla hair type of the grain; presence or absence of hairs in the ventral furrow of the grain; for male sterile parent lines as components of hybrid varieties: presence or absence of pollen production.

5.5 Plots are sown from the submitted seed in each year of test as follows:

Number of plots:	2
Total Number of Plants Examined/Variety:	2000 (approx)
No. of plants /m <sup>2</sup>	200-300
Time of sowing (winter Barley)	October - November
Time of sowing (spring Barley)	February - April

5.6 In the case where two samples have been submitted (ie selected seed and bulk seed), all testing is conducted on the selected seed sample. A sample of 100g will be withdrawn from the bulk seed and sown side by side to the selected seed for authentication in the first year of test.

5.7 In the case of winter varieties, an additional plot is sown in late April during the first year of tests to examine the uniformity of the vernalisation response (characteristic 'Seasonal Type'):

Number of plots:	1
Total Number of Plants Examined/Variety:	minimum 500 plants (approx)
No. of plants /m <sup>2</sup>	200-300
Time of sowing (winter wheat)	Late April

5.8 At the end of the first recording year, reference varieties which are most similar to the candidate varieties are identified and may be sown in the second year of test.

5.9 At the end of the second year of tests, candidate varieties that are still not distinct may be grown in additional direct comparison plots. This requires approval from APHA, and an additional charge will be made to the applicant.

## 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 plant or plants resulting from an adverse reaction to husbandry practice are recorded but excluded from analysis.

6.3 In the first recording year, characters, as indicated in Section D, are recorded on all varieties in test and the data analysed to assess uniformity of the candidate varieties and to determine the most similar reference varieties for each candidate. (For details see Section G).

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

6.5 If the Test Centre notices unusual or novel characters in candidate varieties they may be noted and a photographic record taken.

## 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 growing season. 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 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 tests 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 of characteristics.

## 4 Organisation

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

## 5 DUS Characteristics to be Assessed

### 5.1 Routine Characteristics

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

Note:

\* a characteristic which must be examined according to the CPVO protocol in force and/or 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

Number of plants or sample size for assessment

A Sample size of 100 (See Section G 6.6)

B Sample size of 2000

## 5.1 Barley Characteristics Routinely Recorded in DUS Tests

CPVO TP 019/5	UPOV TG/9/11	Character	Material examined	Number of plants or sample size for assessment	Method of assessment and recording	States of expression	D Method and Minimum Distance required	U Method: standard applied
1	1	<b>Kernel: colour of aleurone layer</b>	Submitted seed	A	VG	1 whitish 2 light grey blue 3 dark grey blue 4 purple 5 black	2 states	1% @95%
2	2*	<b>Plant: Growth habit</b>	Field grown plot	B	VG	1 erect 3 semi-erect 5 intermediate 7 semi-prostrate 9 prostrate	2 - 3 states	0.1% @95%
3	3	<b>Plant: intensity of green colour</b>	Field grown plot	B	VG	1 light 2 medium 3 dark	2 states	0.1% @95%
4G	4*	<b>Lowest Leaves: hairiness of leaf sheaths</b>	Sub sample in field grown plot	A	VG	1 absent 9 present	1 state	1% @95%
5	5*	<b>Flag leaf: intensity of anthocyanin colouration of auricles</b>	Field grown plot	B	VG	1 absent or very weak 3 weak 5 medium 7 strong 9 very strong	2 states	0.1% @95%
6	6	<b>Flag leaf: attitude</b>	Field grown plot	B	VG	1 erect 3 semi-erect 5 horizontal 7 semi-reflexed 9 reflexed	2 states	0.1% @95%
7	7*	<b>Time of ear emergence: (first spikelet visible on 50% of ears)</b>	Field grown plot	B	MG	1 very early 3 early 5 medium 7 late 9 very late	2 days	0.1% @95%

CPVO TP 019/5	UPOV TG/9/11	Character	Material examined	Number of plants or sample size for assessment	Method of assessment and recording	States of expression	D Method and Minimum Distance required	U Method: standard applied
8	8	<b>Flag leaf: glaucosity of sheath</b>	Field grown plot	B	VG	1 absent or very weak 3 weak 5 medium 7 strong 9 very strong	2 states	0.1% @95%
9	9*	<b>Awns: anthocyanin colouration of tips</b>	Field grown plot	B	VG	1 absent or very weak 3 weak 5 medium 7 strong 9 very strong	2 states	0.1% @95%
10	10*	<b>Ear: glaucosity</b>	Field grown plot	B	VG	1 absent or very weak 3 weak 5 medium 7 strong 9 very strong	2 states	0.1% @95%
11	11	<b>Ear: attitude</b>	Field grown plot	B	VG	1 erect 3 semi-erect 5 horizontal 7 semi-drooping 9 drooping	2 states	0.1% @95%
12	12	<b>Grain: anthocyanin colouration of nerves of lemma</b>	Field grown plot	B	VG	1 absent or very weak 3 weak 5 medium 7 strong 9 very strong	2 states	0.1% @95%
13	13*	<b>Plant: length (stem, ear and awns)</b>	Field grown plot	B	MG	1 very short 3 short 5 medium 7 long 9 very long	2 states	0.1% @95%
14G	14*	<b>Ear: number of rows</b>	Field grown plot	B	VG	1 two 2 six	1 state	0.1% @95%
15G	15*	<b>Ear: development of sterile spikelets</b>	Field grown plot	B	VG	1 none or rudimentary ("deficiens") 2 full	1 state	0.1% @95%
16	16*	<b>Sterile spikelet: attitude (in mid-third of ear)</b>	Samples from field grown plot	A	VG	1 parallel 2 parallel to divergent 3 divergent	2 states	1% @95%
17	17*	<b>Ear: shape</b>	Samples from field grown plot	A	VG	1 strongly tapering 2 slightly tapering 3 parallel 4 fusiform	2 states	1% @95%



CPVO TP 019/5	UPOV TG/9/11	Character	Material examined	Number of plants or sample size for assessment	Method of assessment and recording	States of expression	D Method and Minimum Distance required	U Method: standard applied
18	18*	<b>Ear: density</b>	Samples from field grown plot	A for distinctness B for uniformity	VG	3 sparse 5 medium 7 dense 9 very dense	2 states	0.1% @95%
19	19	<b>Ear: length (excluding awns)</b>	Samples from field grown plot	A	VG	3 short 5 medium 7 long	2 states	1% @95%
20	20*	<b>Awn: length (compared to ear)</b>	Samples from field grown plot	A	VG	1 very short 3 short 5 medium 7 long 9 very long	2 states	1% @95%
21	21	<b>Rachis: length of first segment</b>	Samples from field grown plot	A	VG	3 short 5 medium 7 long	2 states	1% @95%
22	22	<b>Rachis: curvature of first segment</b>	Samples from field grown plot	A	VG	1 absent or very weak 3 weak 5 medium 7 strong	2 states	1% @95%
23	23*	<b>Median spikelet: length of glume and its awn relative to grain</b>	Samples from field grown plot	A	VG	1 shorter 2 equal 3 slightly longer 4 longer	1 state	1% @95%
24G	24*	<b>Grain: rachilla hair type</b>	Samples from field grown plot	A	VG	1 short 2 long	1 state	1% @95%
25	25	<b>Grain: spiculation of inner lateral nerves of dorsal side of lemma</b>	Samples from field grown plot	A	VG	1 absent or very weak 3 weak 5 medium 7 strong	2 states	1% @95%
26	26*	<b>Grain: type</b>	Samples from field grown plot	A	VG	1 non-husked 9 husked	1 state	1% @95%

CPVO TP 019/5	UPOV TG/9/11	Character	Material examined	Number of plants or sample size for assessment	Method of assessment and recording	States of expression	D Method and Minimum Distance required	U Method: standard applied
27G	27*	<b>Grain: hairiness of ventral furrow</b>	Samples from field grown plot	A	VG	1 absent 9 present	1 state	1% @95%
28	28	<b>Lemma: shape of base</b>	Samples from field grown plot	A	VG	1 non bevelled 2 bevelled	1 state	1% @95%
29G	29	<b>Seasonal type</b>	Field grown plot	Winter and alternative types: 1000 plant plot test sown in late spring Spring types: TQ declaration	VG	1 winter type 2 alternative type 3 spring type	1 state	1% @95%

## 5.2 Special Category Characteristics

These characters should only be used as a complement to confirm other morphological or physiological differences.

CPVO TP 019/5	UPOV TG/19/1	Character	Material examined	Number of plants or sample size for assessment	Method of assessment and recording	States of expression (SDS PAGE method)	D Method and Minimum Distance required	U Method: standard applied
30	30	<b>D-Hordein composition: allele expression at locus Hor-3</b>	Submitted seed	20 grains for Distinctness 100 grains for Uniformity	Visual score	1 – band 34 2 – band 33 3 – band 35 4 – band 32.5 5 – band 32	1 state	1% @95%  see note below
31	31	<b>C-Hordein composition: allele expression at locus Hor-1</b>	Submitted seed	20 grains for Distinctness 100 grains for Uniformity	Visual score	1 - bands 62 +65 +68 2 - bands 62 +65 +66 + 68 3 - bands 65 +68 4 - bands 66.5 +71 5 - bands 61.5 +66.5 +71 6 - bands 65 7- bands 60 +67.5 +68.5 8 - bands 61 +65 +68 +73 9 - bands 69 +72 10 - bands 64 +66.5 11 - bands 67 +71 12 - bands 65 +68 +69 +70 13 - bands 61.5 +68 +71 14 - bands 65 +67.5	1 state	1% @95%  see note below

CPVO TP 019/5	UPOV TG/19/1	Character	Material examined	Number of plants or sample size for assessment	Method of assessment and recording	States of expression (SDS PAGE method)	D Method and Minimum Distance required	U Method: standard applied
32	32	<b>B-Hordein composition: allele expression at locus Hor-2</b>	Submitted seed	20 grains for Distinctness 100 grains for Uniformity	Visual score	1 - bands 79 +86 +88 +100 2 - bands 79 +88 +91 +95 +97 +101 3 - bands 79 +91 +92 +95 +97 +101 4 - bands 75 +82 +87 +91 +97 5 - bands 79 +86 +88 +97 +101 6 - bands 78 +84 +95 + 101 7 - bands 79 +90 +91 +94 +100 8 - bands 78 +86 +91 +95 +100 9 - bands 79 +82 +88 +91 +92 +101 10 - bands 76 +79 +86 +88 +100 11 - bands 79 +86 +89 +92 +95 +101 12 - bands 79 +95 +101 13 - bands 78 +89 +92 + 101 14 - bands 75 +78 +79 +81 +89 +101 15 - bands 75 +78 +79 +81 +83 +86 +88 +94 +95 +100 16 - bands 81 +84 +88 +90 +101 17 - bands 75 +78 +79 +81 +83 +86 18 - bands 82 +88 +100 19 - bands 81 +100 20 - bands 75 +79 +83 +89 +91 21 - bands 79 +84 +92 22 – bands 79 +91 +92 23 – bands 75 +79 +91 +92 +95 +97 +101 24 – bands 75 +79 +90 +94 +99 25 – bands 79 +(83-85) +(89-91) + (94-96) +102	1 state	1% @95%  see note below

Note – allowance is made for the presence of biotypes

## 5.3 CPVO Approved Additional Characteristics

The following table summarises the additional characteristics which have been approved by the CPVO for Barley.

Type of expression	Characteristic	Growth Stage	Method of observation	States of expression	Example varieties	Note
Qualitative	Production of pollen (male sterility)	60 – 65	Visual score	absent present	FM 99-18 MT 99-18	1 9

5.4 New Additional DUS Characteristics Applicants can suggest new additional characters on the TQ 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 not 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 seed of the variety. In the case of a 'selected seed' sample and a 'bulk seed' sample being submitted, the 'selected seed' sample will be considered to be the definitive sample until the bulk sample has been authenticated (Section C). Subject to meeting the required quality standards (see Section B) the seed is dried and placed in storage under controlled and monitored refrigerated conditions as part of the official reference collection.

4.2 If during the normal tests there is any evidence that seed is deteriorating in storage, or that stocks are under 500g, a request will be made to the maintainer asking for replacement seed (2 kg) of the variety. This replacement seed must be authenticated against the definitive seed.

4.3 Plots will be established from any replacement reference seed sample to be authenticated and compared visually with the definitive seed over a maximum of two recording seasons. Plots must be examined through all the growth stages from early growth habit to full harvest ripeness. If the new seed sample cannot be visually distinguished from the reference seed it will be accepted as representing the variety. It will then be considered as the definitive seed and substituted for the existing definitive seed in the reference collection.

4.4 In the event of the replacement sample not meeting the required acceptance standards, an additional replacement sample will be requested. If the additional replacement sample does not meet the acceptance criteria set out in 4.3, the variety will be deleted from the reference collection.

## **5 Procedures for VCU Seed Stock Authentication**

5.1 A representative sub-sample of seven grains from the VCU seed submission are compared to a representative sub-sample of seven grains from the DUS seed submission (definitive seed) by electrophoresis using the SDS PAGE method. If the VCU seed sample tested by the electrophoresis method does match the DUS seed, it will be considered to represent the variety.

5.2 If the VCU seed sample does not show the same banding pattern as the DUS seed sample a further electrophoresis test will be carried out. If the VCU seed sample still does not match the DUS seed, side-by-side field plots of the two samples will be established and compared visually from early growth habit to full harvest ripeness.

5.3 If the VCU plot does not differ from the DUS plot in the comparison of field sown plots the VCU seed will be considered to represent the variety.

5.4 If the VCU plot can be visually distinguished from the definitive stock in the authentication plots then it will not be accepted as representing the variety.

## **6 Procedures for the Inclusion of New Common Knowledge Varieties into the Reference Collection**

6.1 When a new variety enters into common knowledge such that it must be included in the reference collection, a request will be sent by the Test Centre to the Testing Authority which has added this variety to its National list for the supply of at least 200g of seed of the definitive sample. This seed will then be used to validate a larger sample of seed from the breeder or the VCU seed sample.

## **7 Release of Reference Samples for Authorised Purposes**

7.1 A maximum of 200g of seed of reference samples can be supplied by the Test Centre, on request, to UK, EU and UPOV, DUS Testing Authorities and UK, EU 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.

7.2 Provision of reference samples, other than in 7.1, 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 Barley entered for National List trials and/or PBR.

## 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 requirements will be met.

3.2 All new additional characteristics must be authorised by the NLSC and CPVO.

## 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 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 or CPVO (as appropriate) 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, D5.2 or D5.3 as appropriate.



# Section G – Procedures for DUS Decisions

## 1 Purpose

1.1 This section sets out the standards used to assess distinctness, uniformity and stability of varieties of Barley.

## 2 Scope

2.1 These procedures apply to all varieties of Barley entered for National List and/or Plant Breeders' Rights tests and those being tested on behalf of 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 report in accordance with these procedures and for ensuring that they are in accordance with CPVO protocols or UPOV guidelines as appropriate.

## 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 TG/1/3, varieties can be considered distinct where they have a different expression in a grouping character e.g. seasonal type; presence or absence of hairs on the lower leaf sheaths; presence or absence of sterile spikelets in the ear; number of rows in the ear; rachilla hair type of the grain; presence or absence of hairs in the ventral furrow of the grain; for male sterile parent lines as components of hybrid varieties: presence or absence of pollen production.

5.2 The standard applied for distinctness over two years of test is a clear difference of one or two states in the expression of a characteristic in accordance with the table of characteristics given above in Section D.

5.3 If distinctness is assessed using the 2 x 1% criterion, the varieties need to be significantly different in the same direction at the 1% level in at least two out of three years in one or more measured characteristics. The tests in each year are based on Student's two-tailed t-test of the differences between variety means with standard errors estimated using the residual mean square from the analysis of the variety x replicate plot means.

5.4 Where varieties are grown in close proximity under the same conditions, and a direct comparison can be made, distinctness can be determined on the basis of visual observation. Characters are recorded using notes to represent states of expression (See Section D). In these circumstances the basis for distinctness will be clearly recorded.

## 5.5 Hybrids

Distinctness follows the principle of “hybrid first” i.e. if the final hybrid variety is not distinct then distinctness is examined by testing the parent lines – either the CMS (female parent) or the Restorer (pollen donor/male parent) must be clearly distinguishable from the respective male or female parent of the non-distinct hybrid variety. Hybridity may be used as a grouping character based on the TQ declaration made by the applicant. CMS female parent lines will only be compared to other CMS lines. Pollen production – absence or presence may also be used as a UK grouping character. It is tested by growing the male sterile parent line in isolated seed plots of approx. 2000 plants.

## 6 Uniformity

6.1 Uniformity is assessed for all characteristics used to establish Distinctness.

6.2 Uniformity is based on the assessment of off-types (variants) for visually observed characters.

6.3 The assessment of off-types is undertaken in both test cycles and the total should not exceed the number allowed using the population standards detailed below. Care is taken to ensure that the plants that are counted are not the result of any non-genetic factors such as environment, pest and disease.

6.4 In a sample size of 2,000 (characters marked as “B” in Section D), a population standard of 0.1% and an acceptance probability of at least 95% should be applied. For example in a sample of 2000 plants, 5 off-types are allowed.

6.5 In a sample size of 100 (characters marked as A in Section D), a population standard of 1% and an acceptance probability of at least 95% should be applied. For example, in a sample of 100 plants or parts of plants, 3 off-types are allowed.

6.6 For characters marked as “A” in Section D (with the exception of characteristic 1), the assessment of uniformity can be carried out in two stages. In the first stage, 20 plants or parts of plants are examined. If no off-types are observed the variety is declared uniform. If more than three off-types are found the variety is declared not to be uniform. If one to three off-types are observed, a further 80 plants or parts of plants should be examined.

6.7 The following uniformity standards apply to hybrids and their progenitor lines:

6.7.1 Hybrid B characters 10% @ 95% probability  
A characters 10% @ 95% probability

The maximum permissible number of off-types for B characters will be 225 plants or parts of plants in a sample of 2000. The sample size for assessment can be reduced to 200 plants. In this case, a maximum of 27 off-types are allowed. The maximum permissible number of off-types for A characters will be 15 plants or parts of plants in a sample of 100 plants or parts of plants.

6.7.2. Male sterile parent B characters 0.2% @ 95% probability  
A characters 1% @ 95% probability

The maximum permissible number of off-types for B characters will be 8/2000 and the maximum permissible for A characters will be 3/100.

6.7.3. Male sterile single cross hybrids B characters 0.5% @ 95% probability (used as parent in a 3-way hybrid) A characters 1% @ 95% probability

The maximum permissible number of off-types for B characters will be 15/2000 and the maximum permissible for A characters will be 3/100.

6.7.4. The pollinator and restorer lines are usually inbred lines and will be tested as conventional self-pollinating varieties (see 6.4. and 6.5 above).

## 6.8 Resubmissions

For all varieties, except hybrid varieties, a resubmission of plant material may be allowed for the second growing cycle if in the first growing cycle the number of off-types did not exceed 15 plants in a sample size of 2000 plants (population standard of 0.5% with an acceptance probability of at least 95%) or 9 plants or parts of plants in a sample size of 100 (population standard of 5% with an acceptance probability of at least 95%).

## 7 Stability

7.1 A variety is considered sufficiently stable when there is no evidence to indicate that it lacks uniformity or fails to conform to the essential characteristics of its description in different submissions or in different tests.

7.2 Hybrids may be considered to lack stability if there is evidence that their progenitor lines lack uniformity or fail to conform to the essential characteristics of their description.

7.3 For three-way hybrids with segregating progenitor lines, the production and maintenance schemes of all progenitor lines must indicate that the final hybrid (candidate) can, in terms of its genetic constitution, be consistently reproduced in each cycle of propagation.

## 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 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, will be submitted to APHA by the specified date.

# 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, including those being tested for other Member States or the CPVO.

1.3 All varieties on the UK National List including any entered for export only to another Member State.

1.4 All varieties with UK PBR.

1.5 Varieties on the EC Common Catalogue whose seed is known to be certified or marketed in the UK that are listed by countries with comparable climatic conditions to UK.

1.6 All varieties with EU PBR that are listed by countries with comparable climatic conditions to the UK.

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

1.8 Varieties nominated by the authorities concerned where tests are done for other Member States.

1.9 Any varieties nominated by the applicant as being comparable i.e. known to be similar.

1.10 Any other varieties considered to be comparable i.e. known to be similar by the appropriate Test Centre or DUS Centre Group.



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