Animal &
Plant Health
Agency

United Kingdom Variety Testing / Plant Breeders' Rights Technical Protocol for Official Examination of Distinctness, Uniformity and Stability (DUS)

Perennial Ryegrass

Lolium perenne L.

December 2022

Contents

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 Perennial Ryegrass entered for Variety Listing (VL) and/or Plant Breeders' Rights (PBR).

2 Scope

2.1 These procedures apply to all varieties of Perennial Ryegrass (*Lolium perenne L*.). 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 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, 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; Scottish Government (SG); the Department of Agriculture, Environment and Rural Affairs (DAERA); and the 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 or the Test Centre.

3.4 The procedures are administered by:

Plant Variety Rights Office for the UK
Animal and Plant Health Agency
Eastbrook
Shaftesbury Road
Cambridge
CB2 8DR Email: pvs.helpdesk@apha.gov.uk

3.5 Test Centre

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

Herbage DUS Test Centre
Agri-Food and Biosciences Institute (AFBI)
Plant Testing Station
Crossnacreevy
Belfast Tel no 02890 548000
BT6 9SH Fax no 02890 548001

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 technical reasons not to do so which can be justified by the Test Centre.

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 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
Perennial Ryegrass VCU Protocol	United Kingdom Variety List Trials: Protocol and Procedures for Examining the Value for Cultivation and Use (VCU) of Perennial Ryegrass.
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).
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 TG/33/7	Combined Over-Years Criterion for Distinctness (COYD) and Uniformity (COYU), (Revision of document TC/30/4) (09.12.1997).
UPOV TG/4/8	Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, Perennial Ryegrass (09.04.2008).
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 Varieties and Seeds (2001) 14 1-14	The potential for management of reference collections in herbage variety registration trials using a cyclic planting system for reference varieties.
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 website https://www.gov.uk/national-lists-of-agricultural-and-vegetable-crops

4.2 The procedures for the submission of Variety Listing 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 GOV.UK web site at https://www.gov.uk/national-lists-of-agricultural-and-vegetable-crops

4.3 Applicants must note in the TQ submitted with the application any additional characteristics which may require examinations that are listed in the DUS characteristics Section D, 5.2 or 5.3 (an additional fee may be required). Applications received after this date may be considered for inclusion in the current year's tests and trials on a case-by-case basis.

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 normally be refused. Instructions for the delivery of seed will be made available to applicants by APHA https://www.gov.uk/national-lists-of-agricultural-and-vegetable-crops

6 Seed Quality Requirements

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

6.2 The seed must not be chemically treated. Seed treatment, where appropriate, 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

Perennial Ryegrass - diploid	2400 g*
Perennial Ryegrass - tetraploid	3200 g"

* Comprises 1500g for DUS tests and 900g for VCU trials.

"Comprises 2000g for DUS tests and 1200g for VCU trials.

The DUS and VCU seed must be supplied as one lot.

7.2 Year 2 and Further Year Submissions

A sample of 22g of diploid and 33g of tetraploid seed will be withdrawn from VCU submissions in year 2 and any further years to authenticate the submission. Applicants should refer to Trial Procedures for Official Examination of Value for Cultivation and Use (VCU) Perennial Ryegrass for seed requirements.

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. This must be agreed in advance with APHA and the test centre.

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
- Breeder's reference number or name
- AFP number (if known)
- Type of seed (DUS only/combined submission of DUS and VCU for year 1 sowings).
- Quantity of seed
- Whether it is a parental line.

8.2 All packages of GM material must be labelled clearly 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 Perennial Ryegrass (*Lolium perenne L*).

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 normally not had a Perennial Ryegrass seed crop in the previous five years but may be less where it has been determined the risk is negligible.

5.2 Field husbandry should follow best 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. The National List and Seeds Committee (NLSC) must be informed on any proposed changes to the number of cycles.

5.4 From information given in the Technical Questionnaire the candidate variety may be grown in a single-spaced plant test and compared with varieties which are in the same classification for the following characters, ploidy and utilisation type – forage or amenity.

5.5 The tests are carried out using a randomised block design, with a plot of each variety present in every block as follows

No. of blocks	6
No. of plants per block	10
Hence, No. of plants per variety	60
Plant spacing	75 cm (approx.)

The plots are arranged in the order of the sowing list in the first block. The plots are fully randomised within each of the other five blocks.

5.5 Seed is sown singly under glass into multipots in March/April. After establishment, the plants are moved outdoors for hardening off and transplanted in the field in July to provide single spaced plants according to a plan produced by the Test Centre. Varieties are coded by the Test Centre.

5.6 At the end of the second and third recording years in September/October, any candidate varieties with serious distinctness problems are sown out in close comparison plots. These are row plots grown alongside relevant problem varieties for examination during the subsequent years.

5.7 For glasshouse seedling tests, a trial with thirty established plants of each variety is replicated in time by being sown at weekly intervals for six weeks, so giving 180 plants per variety in total. The plants in each trial are fully randomised.

5.8 Recordings are taken on each trial after approximately 8-12 weeks, depending upon the growth stage. Characters recorded are those agreed with 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 plants 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 D5.1, are measured on all varieties and the data analysed to assess uniformity 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 of the candidate variety. (For details see Section G).

6.5 In the third 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 and second year, used to assess distinctness and uniformity of the candidate variety. (For details see Section G).

6.6 If the Test Centre notices unusual or novel characters in candidate varieties they must be noted at any time and a photographic record taken.

7 Communication 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 tests 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 should normally be three independent growing cycles although varieties may be determined DUS after two years of tests. Shorter periods may be applied for assessment of additional characteristics. Proposed changes to the number of growing cycles may 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 UPOV Guidelines.

G a grouping characteristic.

D a characteristic used in the variety description.

5.1 Perennial Ryegrass Characteristics Routinely Recorded in DUS Tests

CPVO TP/4/2	UPOV TG/4/8	Character	Sample source (Material examined)	Number of plants or sample size for	Method of assessment and recording	States of expression	D Method Minimum difference required UPOV TC33/7	U Method UPOV UPOV
1GD	1*	**Plant: ploidy**	Single spaced plant test (DUS plot)	60	TQ declaration/ laboratory	2=Diploid 4=Tetraploid	Ploidy difference	Observation
2D	2	**Plant: vegetative growth habit (without vernalization)**	DUS plot	60	Visually scored	1=erect 3=semi-erect 5=medium 7=semi-prostrate 9=prostrate	COYD @1%	COYU @ 0.1%
3D	5	**Leaf: intensity of green colour (without vernalization)**	DUS plot	60	Visually scored	1=very light 3=light 5=medium 7=dark 9=very dark	COYD @1%	COYU @ 0.1%
4D	6	**Plant: width (after vernalization)**	DUS plot	60	Measured	1=very narrow 3=narrow 5=medium 7=wide 9=very wide	COYD @1%	COYU @ 0.1%
5D	7	**Plant: vegetative growth habit (after vernalization)**	DUS plot	60	Visually scored	1=erect 3=semi-erect 5=medium 7=semi-prostrate 9=prostrate	COYD @1%	COYU @ 0.1%
6D	8	**Plant: height (after vernalization)**	DUS plot	60	Measured	1=very short 3=short 5=medium 7=tall 9=very tall	COYD @1%	COYU @0.1%
7D		**Leaf: intensity of green colour (after vernalization)**	DUS plot	60	Visually scored	1=very light 3=light 5=medium 7=dark 9=very dark	COYD @1%	COYU @0.1%
9D	10	**Plant: tendency to form inflorescences (without vernalization)**	DUS plot	60	Visually scored	1=absent or very weak 3=weak 5=medium	COYD @1%	COYU @0.1%
10D	11*	**Plant: time of inflorescence emergence (after vernalization)**	DUS plot	60	Visually scored, and time recorded	1=very early 3=early 5=medium 7=late 9=very late	COYD @1%	COYU @0.1%
11D	12	**Plant: natural height at inflorescence emergence**	DUS plot	60	Measured	1=very short 3=short 5=medium 7=tall 9=very tall	COYD @1%	COYU @0.1%

CPVO TP/4/2	UPOV TG/4/8	Character	Sample source (Material examined)	Number of plants or sample size for	Method of assessment and recording	States of expression	D Method Minimum difference required UPOV	U Method UPOV UPOV TC33/7
12D		**Plant: habit of growth at inflorescence emergence**	DUS plot	60	Computer derived (UK 11/10)	1=erect 3=semi-erect 5=medium 7=semi-prostrate 9=very prostrate	COYD @1%	COYU @0.1%
13D	14*	**Flag Leaf: length**	DUS plot	60	Measured	1=very short 3=short 5=medium 7=long 9=very long	COYD @1%	COYU @0.1%
14D	15*	**Flag Leaf: width**	DUS plot	60	Measured	1=very narrow 3=narrow 5=medium 7=broad 9=very broad	COYD @1%	COYU @0.1%
15D	16	**Flag leaf: length/width ratio**	DUS plot	60	Computer derived (UK 14/15)	1=very low 3=low 5=medium 7=high 9=very high	COYD @1%	COYU @0.1%
16D	17*	**Plant: length of longest stem, inflorescence included(when fully expanded)**	DUS plot	60	Measured	1=very short 3=short 5=medium 7=long 9=very long	COYD @1%	COYU @0.1%
17D	18	**Plant: length of upper internode**	DUS plot	60	Measured	1=very short 3=short 5=medium 7=long 9=very long	COYD @1%	COYU @0.1%
18D	19	**Inflorescence: length**	DUS plot	60	Measured	1=very short 3=short 5=medium 7=long 9=very long	COYD @1%	COYU @0.1%
19D	20	**Inflorescence: number of spikelets**	DUS plot	60	Counted	1=very few 3=few 5=medium 7=many 9=very many	COYD @1%	COYU @0.1%
20D	21	**Inflorescence: density**	DUS plot	60	Computer derived (UK 24/31)	1=very lax 3=lax 5=medium 7=dense 9=very dense	COYD @1%	COYU @0.1%

CPVO TP/4/2	UPOV TG/4/8	Character	Sample source (Material examined)	Number of plants or sample size for	Method of assessment and recording	States of expression	D Method Minimum difference required UPOV	U Method UPOV UPOV TC33/7

21D	22	**Inflorescence: length of outer glume on basal spikelet**	DUS plot	60	Measured	1=very short 3=short 5=medium 7=long 9=very long	COYD @1%	COYU @0.1%
22D	23	**Inflorescence: length of basal spikelet excluding awn**	DUS plot	60	Measured	1=very short 3=short 5=medium 7=long 9=very long	COYD @1%	COYU @0.1%

5.2 UK Approved Additional Characteristics (Non CPVO approved)

The following table summarises UK approved characteristics which have been accepted by the NLSC for DUS assessment. These characteristics can be derived from the primary characteristics without incurring additional examination costs. *NB UK Character 11 (Plant: width at inflorescence emergence) is required to allow the calculation of CPVO Character 12 (Plant: habit of growth at inflorescence emergence).*

CPVO TP/4/2	UPOV TG/4/8	UK	Character	Sample source (Material examined)	Number of plants or sample size for assessment	Method of assessment and recording	States of expression	D Method Minimum difference required UPOV TC33/7	U Method UPOV UPOV TC33/7
N/A	N/A	60	**Plant: natural height (after vernalization)**	DUS plot	60	Measured	1=very short 3=short 5=medium 7=tall 9=very tall	COYD @1%	COYU @0.1%
N/A	13	11	**Plant: width at inflorescence emergence**	DUS plot	60	Measured	1=very narrow 3=narrow 5=medium 7=wide 9=very wide	COYD @1%	COYU @0.1%
N/A	N/A	51	**Plant: vegetative spring development**	DUS plot	60	Computer derived (UK 5-60)	1=very little 3=little 5=medium 7=much 9=very much	COYD @1%	COYU @0.1%
N/A	N/A	52	**Plant: vegetative growth habit**	DUS plot	60	Computer derived (UK 5/70)	3=prostrate 5=medium 7=erect	COYD @1%	COYU @0.1%
N/A	N/A	54	**Plant: vegetative attitude in spring**	DUS plot	60	Computer derived (UK 5/60)	1=very prostrate 3=prostrate 5=medium 7=erect 9=very erect	COYD @1%	COYU @0.1%
CPVO TP/4/2	**UPOV TG/4/8**	**UK**	**Character**	**Sample source (Material examined)**	**Number of plants or sample size for assessment**	**Method of assessment and recording**	**States of expression**	**D Method Minimum difference required UPOV TC33/7**	**U Method UPOV UPOV TC33/7**

N/A	N/A	71	**Plant: vegetative shape in spring**	DUS plot	60	Computer derived (UK 60/70)	1=very compact 3=compact 5=medium 7=spreading 9=very spreading	COYD @1%	COYU @0.1%
N/A	N/A	103	**Plant: seasonal width**	DUS plot	60	Computer derived (UK √ (11*70))	1=very narrow 3=narrow 5=medium 7=wide 9=very wide	COYD @1%	COYU @0.1%
N/A	N/A	39	**Flag leaf: size**	DUS plot	60	Computer derived (UK √(14*15))	1=very small 3=small 5=medium 7=large 9=very large	COYD @1%	COYU @0.1%
N/A	N/A	101	**Inflorescence: total basal spikelet length**	DUS plot	60	Computer derived (UK √ (24*35))	1=very short 3=short 5=medium 7=long 9=very long	COYD @1%	COYU @0.1%
N/A	N/A	102	**Inflorescence: spikelet protuberance**	DUS plot	60	Computer derived (UK 35-34)	1=very little 3=little 5=medium 7=much 9=very much	COYD @1%	COYU @0.1%
N/A	N/A	107	**Inflorescence: glume span**	DUS plot	60	Computer derived (UK 35/34)	1=very small 3=small 5=medium 7=large 9=very large	COYD @1%	COYU @0.1%
N/A	N/A	108	**Plant: volume**	DUS plot	60	Computer derived (UK √ (11*10))	1=very small 3=small 5=medium 7=large 9=very large	COYD @1%	COYU @0.1%
N/A	N/A	117	**Inflorescence: total length of spikelets**	DUS plot	60	Computer derived (UK 31*35)	1=very small 3=small 5=medium 7=large 9=very large	COYD @1%	COYU @0.1%
N/A	N/A	118	**Inflorescence: total length of glumes**	DUS plot	60	Computer derived (UK 31*34)	1=very small 3=small 5=medium 7=large 9=very large	COYD @1%	COYU @0.1%
N/A	N/A	25D	**Inflorescence: awns**	DUS plot	60	Observations	0=absent 1=present	For description purposes only	N/A

CPVO TP/4/1	UPOV TG/4/8	UK	Character	Sample source (Material examined)	Number of plants or sample size for	Method of assessment and recording	States of expression	D Method Minimum difference required	U Method UPOV UPOV TC33/7
N/A	N/A	124D	**Seedling: fluorescence**	Laboratory test	400	Observations	0=absent 1=present	For description purposes only	N/A

5.3 Approved Additional Characteristics

The following table summarises the additional characteristics which have been approved by the NLSC and can be examined at the request of the applicant where necessary to establish Distinctness. A fee may be charged for examination of these characteristics as advised by APHA.

CPVO TP/4/2	UPOV TG/4/	UK	Character	Sample source (Material examined)	Number of plants or sample size for	Method of assessment and recording	States of expression	D Method Minimum difference required	U Method UPOV UPOV TC33/7
N/A	N/A	22	**Plant: tendency to form inflorescences in the aftermath**	DUS plot	60	Visually scored	1=very little 3=little 5=medium 7=much 9=very much	COYD @1%	COYU @0.1%
N/A	N/A	121	**Seedling: tiller Number**	Glasshouse trial	180	Measured	1=none 3=small 5=medium 7=large 9=very large	ANOVA (t-test) @1% [1]	F test @ 1%[2]
N/A	N/A	122	**Seedling: habit of growth**	Glasshouse trial	180	Visually scored	1=prostrate 3=semi-prostrate 5=medium 7=semi-erect 9=erect	ANOVA (t-test) @1% [1]	F test @ 1%[2]
N/A	N/A	123	**Seedling: width of vegetative leaf**	Glasshouse trial	180	Measured	1=very narrow 3=narrow 5=medium 7=wide 9=very wide	ANOVA (t-test) @1% [1]	F test @ 1%[2]

Note [1] Variety means are compared using trial X variety interaction as an estimate of error variance.

[2] The within trial variance of the candidate variety, averaged over trials, is compared with that of a control variety.

Electrophoresis

In *Lolium perenne* L., the composition of seed proteins can be used as an additional characteristic for establishing distinctness, uniformity and stability on request of the applicant under the following conditions:

- in case of distinctness assessment with COYD, if the difference is significant on a level between 1% and 5% for at least one of the CPVO characteristics mentioned in chapter 5.

- in case of distinctness assessment on the basis of notes, if the difference is at least 1 note in 2 out of 3 years.

Distinctness between two varieties must not be established on the basis of seed protein polymorphisms alone. If electrophoresis is used for testing of distinctness, the same population standard and the same acceptance probability as for other characteristics should be applied for the assessment of uniformity.

Electrophoresis characteristics with a lack of uniformity shall not be taken into account for the assessment of distinctness.

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 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 DUS seed sample submitted with the successful or pending application is considered to be the definitive stock of the variety. Subject to meeting the required certification standards (see Section B) a small portion of the seed is sown for observations and measurements. The remainder is dried and stored 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 a seed stock is deteriorating in storage, or that stocks are less than 100 grams, a request should be made to the maintainer asking for a replacement stock (1500g diploid varieties; 2000g) of the variety. This replacement stock must be authenticated against the definitive reference sample. Plots will be established from any replacement reference seed sample to be authenticated and compared with the definitive stock over a maximum of two recording seasons.

4.3 If the replacement seed sample meets the required standard of no significant (P=0.01) differences in the first year of test using a within-year block by varieties analysis of variance of plot means or no significant (P=0.01) differences over two years in a COYD with MJRA analysis (see associated document UPOV TC/33/7 for details) it will be accepted as representing the variety. It may then be accepted as definitive and substituted for the existing definitive stock in the reference collection.

4.4 A replacement sample or additional replacement sample will be considered sufficiently uniform after one year of test, if the standard deviations of the primary measured characters are not significantly greater at the 1% (P=0.01) significance level than that of the mean standard deviations of the control varieties. A replacement sample or additional replacement sample will be considered sufficiently uniform after two years of test if for all primary measured characters, the combined over years uniformity (COYU) is not significantly greater at the 1% (P=0.01) significance level than that of the reference varieties.

4.5 In the event of the replacement sample not meeting the required acceptance standards, an additional replacement sample is requested. Plots will be established from any additional replacement seed sample and compared over a maximum *of* two recording seasons. 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 and the Variety Lists reviewed.

5 Procedures for VCU Seed Stock Authentication

5.1 Evidence will be requested from the breeder of the relationship between the VCU seed sample and the definitive DUS seed sample. Plots will be established from any VCU seed sample to be authenticated and compared visually with the definitive stock over the recording season.

5.2 The plots must be examined from establishment, through flowering to maturity.

5.3 If the new seed sample cannot be visually distinguished from the reference stock it will be accepted as representing the variety.

5.4 If the VCU seed sample can be visually distinguishable from the definitive stock in the authentication plots then it will not be accepted as representing the candidate 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 Variety List for the supply of at least 50g of seed of the definitive sample. This seed will then be used to validate a larger sample of seed from the breeder. (The amount of seed requested will be 1500g for diploid varieties and 2000g for tetraploid (see B7.1.)) The standards for this validation will be as for VCU seed stock authentication (see E6).

7 Release of Reference Samples for Authorised Purposes

7.1 A maximum of 50g 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, provided the recipient is 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 Perennial Ryegrass entered for Variety Listing and/or PBR trials.

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 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 assessed.

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 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.

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 5.1 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 Perennial Ryegrass.

2 Scope

2.1 These procedures apply to all varieties of Perennial Ryegrass (*Lolium perenne L.*)

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.

4.2 A system of cyclic planting of reference varieties in two years out of every three years is used, with the data for the missing year compensated for by the use of historic data from two earlier years. Please see associated document Plant Varieties and Seeds (2001) 14,1-14 for details.

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 and utilisation type.

5.2 The standard applied for distinctness over two years of test is a significant difference at 1% (P = 0.01) significance level in at least one character in a combined over years distinctness (COYD) with Modified Joint Regression (MJRA) analysis. Please see associated document UPOV TC/33/7 for details.

5.3 The standard applied over three years of test is a significant difference at the 1% (P = 0.01) in at least one character in a combined over years distinctness (COYD) with Modified Joint Regression (MJRA) analysis. Please see associated document UPOV TC/33/7 for details.

5.4 A two-tier system is used for assessing distinctness. This determines the characteristics for which a variety must also be uniform. The varieties are examined first for distinctness using only the primary measured characters. The varieties which are not distinct are then re-examined using secondary computer-derived characteristics. A variety must be uniform in all primary measured characters. However, if a secondary computer-derived characteristic is necessary for distinctness in a variety, then the variety must also be uniform in that characteristic.

5.5 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 assessed for all characteristics used to establish distinctness.

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.

6.3 After the variants have been excluded, the characteristics listed in Section D5 are used to assess the uniformity of the remaining plants, according to the methods of Combined Over Years Uniformity (COYU) analysis described in associated document UPOV TC/33/7.

6.4 A variety can be considered sufficiently uniform after two years of tests if, for all primary measured characters and any secondary computer-derived characters necessary for distinctness, the combined over years uniformity (COYU) is not significantly greater than that of the reference varieties at the 1% (P=0.01) significance level. In all cases an examination of data from individual years is carried out to investigate the COYU result should this reveal potential uniformity problems.

6.5 A variety is considered sufficiently uniform after three years of tests when, for all primary measured characters and any secondary computer-derived characters required for distinctness, the combined over years uniformity (COYU) is not significantly greater than that of the reference varieties at the 0.1% (P=0.001) significance level. In all cases an examination of the data from individual years is carried out to investigate the COYU result should this reveal potential uniformity problems.

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.

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 Variety 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:

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

1.1.2 All varieties with UK PBR.

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

1.1.4 All varieties protected under National PBR (UPOV contracting parties) with comparable climatic conditions to the UK

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 Test Centre or DUS Centre Group.

1.1.7 Other available comparable varieties in common knowledge.

Appendix 2 – Electrophoresis

The following section contains a characteristic derived by using protein electrophoresis and a description of the method to be used.

1 Introduction

In *Lolium perenne* L., the composition of seed proteins can be used as additional characteristic for establishing distinctness, uniformity and stability on request of the applicant under the following conditions:

- in case of distinctness assessment with COYD, if the difference is significant on a level between 1 % and 5 % for at least one of the characteristics mentioned in SECTION 5.1,
- in case of distinctness assessment on the basis of notes, if the difference is at least 1 note in 2 out of 3 years.

Distinctness between two varieties must not be established on the basis of seed protein polymorphisms alone.

2 Characteristics Derived by Using Electrophoresis

The composition of seed proteins is determined by SDS-polyacrylamide-gel-electrophoresis (SDS-PAGE). The bands are numbered according to their position in the gel. For the purpose of description, the bands are combined in groups. The characteristic is described for the following band ranges.

Band range	Description of the banding pattern
O	1 – 20
P	21 – 40
R	61 – 90
U	141 – 154
V	155 – 190

3 Description of the SDS-PAGE Method for the Detection of Seed Protein Polymorphism in *Lolium Perenne*

3.1 Number of Seeds per Test

A bulked sample of 1.5 g seed material is analysed per variety. This weight equals an amount of approximately 1000 seeds.

3.2 Equipment

Swing mill
Vortex-mixer
Rocking platform shaker
Table centrifuge (min. 6.000 RPM) Cryostat
Power supply with a capacity of at least 400 V and 150 mA providing constant current and constant voltage output

Any suitable vertical electrophoresis system can be used, provided that the gels can be kept at a constant temperature. A minimum running length of the gels of 10 cm is necessary. A gel thickness of no more than 1.5 mm is recommended.

3.3 Chemicals

All chemicals should be of "Analytical Reagent grade" or better.

3.3.1 Chemicals for Extraction of Seed Proteins

Chemical	Abbreviation	Formula
Glycerol		$C_3H_8O_3$
2-Mercaptoethanol	ß-ME	C_2H_6OS
Sodium dodecyl sulphate	SDS	$C_{12}H_{25}NaO_4S$
Hydrochloric acid	HCl	HCl
Tris-(hydroxymethyl)-aminomethane	Tris	$C_4H_{11}NaO_{11}S$

3.3.2 Chemicals for Electrophoresis

Chemical	Abbreviation	Formula
40% Acrylamide solution(w/v)	AA	C_3H_5NO
Ammonium peroxodisulfate	APS, AP	$(NH_4)_2S_2O_8$
2% Bisacrylamide solution (w/v)	BIS	$C_7H_{10}N_2O_2$
Bromphenol blue		$C_{19}H_{10}Br_4O_5S$
Ethanol	EtOH	C_2H_6O
Glycine		$C_2H_5NO_2$
Dodecyl sulfate Sodium salt	SDS	$C_{12}H_{25}NaO_4S$
Hydrochloric acid		HCl
Sucrose		$C_{12}H_{22}O_{11}$
NNN'N'Tetramethylethylenendiamine	TEMED	$C_6H_{16}N_2$

3.3.3 Chemicals for Staining of Proteins

Chemical	Abbreviation	Formula
Coomassie blue G250		$C_{47}H_{48}N_3O_7S2$ x Na
Coomassie blue R250		$C_{45}H_{44}N_3O_7S_2$ x Na
Glacial acetic acid		$C_2H_4O_2$
Glycerol		$C_3H_8O_3$
Methanol	MeOH	CH_3OH
Trichloro acetic acid	TCA	$CHCl_3O_2$

3.4 solutions

3.4.1 Extraction Solutions

No.	Solution	Ingredients	Amount	Remark
3.4.1.1	Extraction buffer	TRIS Distilled water	12.1g Add 100 ml	Adjust to pH 6.8 with HCl
3.4.1.2	Extraction solution A	SDS Extraction buffer (3.4.1.1) Distilled water Glycerol	4.0 g 12.5 ml 24.0 ml 20.0 ml	Prepare daily Warming to 30°C - 40°C to dissolve SDS if necessary
3.4.1.3	Extraction solution B	Extraction buffer A (3.4.1.2) Distilled water	27.0 ml 17.0 ml	Prepare daily
3.4.1.4	Extraction solution C	Extraction buffer B (3.4.1.3) Mercaptoethanol	22.0 ml 1.5 ml	Prepare daily

3.4.2 Electrophoresis Buffers and Gel Preparation Solutions

No.	Solution	Ingredients	Amount	Remark
3.4.2.1	Resolving gel buffer	TRIS Distilled water	75 g add 1000 ml	Adjust to pH 8.9 with HCl, at 8°C stable for 4 months
3.4.2.2	Stacking gel buffer	TRIS Bromphenol blue Distilled water	16 g 100 mg add 1000 ml	Adjust to pH 6.7 with HCl, at 8°C stable for 4 months
3.4.2.3	Resolving gel preparing solution	Resolving gel buffer (3.4.2.1) 40 % Acrylamide solution (3.4.2.5) 2 % Bisacrylamide solution (3.4.2.6) 10 % SDS-Solution	60 ml 33 ml 8.5 ml 1 ml	Prepare daily
3.4.2.4	Stacking gel preparing solution	Stacking gel buffer(3.4.2.2) 40% Acrylamide solution (3.4.2.5) 2% BIS solution (3.4.2.6) Distilled water Sucrose 10% SDS (3.4.2.8)	280 ml 45 ml 73 ml 150 ml 80 g 6 ml	At 8°C stable for 1 month
3.4.2.5	Acrylamide solution	Acrylamide Distilled water	40 g add 100 ml	It is strongly recommended to use a ready to use solution
3.4.2.6	BIS solution	Bisacrylamide Distilled water	2 g add 100 ml	It is strongly recommended to use a ready to use solution
3.4.2.7	APS-solution	Ammoniumperoxodisulfate Distilled water	1 g add 50 ml	Prepare daily.
3.4.2.8	SDS-solution	SDS Distilled water	10 g add 100 ml	Stable for month.
3.4.2.9	Ethanol 20%	Ethanol Distilled water	20 ml add 100 ml	At room temperature stable for 6 month.
3.4.2.10	Electrophoresis buffer Stock solution	TRIS SDS Glycine Distilled water	103 g 20 g 70 g add 1000 ml	Stable for month.
3.4.2.11	Electrophoresis buffer	Electrophoresis buffer Stock solution (3.4.2.10) Distilled water	50 ml add 1000 ml	Prepare daily.

3.4.3 Staining Solutions

No.	Solution	Ingredients	Amount	Remark
3.4.3.1	Coomassie Blue Stock solution	Coomassie Blue G 250 Coomassie Blue R 250 Tap water	0.25 g 0.75 g add 100ml	Stirred for at least 1 h; Shaken very well before use
3.4.3.2	Staining solution	TCA Acetic acid 80% Tap water Methanol Stock solution (3.4.3.1)	240 ml 520 ml 3100 ml 600 ml 90 ml	N/A
3.4.3.3	Glycerol solution	Glycerol Tap water	50 g add 1000 ml	N/A

3.5 Procedure

3.5.1 Preparation of Samples

1.5 g seeds are ground for 2 minutes by 2000 RPM with a swing mill and 3 steel balls. The grist is stored in a 5 ml glass tube.

3.5.2 Extraction of Samples

0.08 g well mixed grist is weighed in 2 ml reaction tubes and mixed with 1 ml extraction solution C (3.4.1.4) by using a vortex mixer. The samples are left for 1 hour at room temperature, suspended by using a vortex mixer and heated for 20 minutes in a water bath by a temperature of 75°C. After cooling in a water bath, the tubes are centrifuged at 10,000 x g for 10 minutes at 4°C.

15 µl of the clarified supernatant is diluted with 70 µl extraction solution B (3.4.1.3). The sample is stored frozen until SDS-PAGE.

3.5.3 Preparation of Gels

3.5.3.1 Preparation of the Gels for SDS-PAGE

The SDS-PAGE is a discontinuous electrophoresis, and each gel consists of resolving gel and stacking gel. Clean and dry gel cassettes are assembled, according to the design of the equipment used.

The resolving gel solution is composed as described in 3.4.2.3 and polymerisation is started by addition of:

100 µl TEMED
5 ml APS solution (1.3.4.2.7).

The gels are carefully poured, avoiding the formation of bubbles. The gel should be poured to a height which leaves room for a 20 mm layer of stacking gel. The gel surface is carefully overlapped with 20% ethanol solution (3.4.2.9) using a syringe. The gel polymerises at room temperature for at least one hour. When the polymerisation is finished, the ethanol solution is removed. The gel surface is rinsed with distilled water and dried with filter paper.

To make the stacking gels the following is mixed under slow stirring:

15 ml stacking gel preparing solution (3.4.2.4)
60 µl TEMED
375 µl APS-solution (3.4.2.7)

The gels are carefully poured, avoiding the formation of bubbles. The height of the stacking gel should be about 2 cm. The well-forming combs are inserted into the liquid gel. The gels are allowed to polymerize at room temperature for about 1 hour. The combs should be removed carefully out of the gel.

3.5.4 Sample Loading

The wells of the gel are carefully rinsed using electrophoresis buffer (3.4.2.11).
For separation of the seed proteins each well is filled with 5 µl extract (see 3.5.2) using a multiple syringe.

3.5.5 Electrophoresis

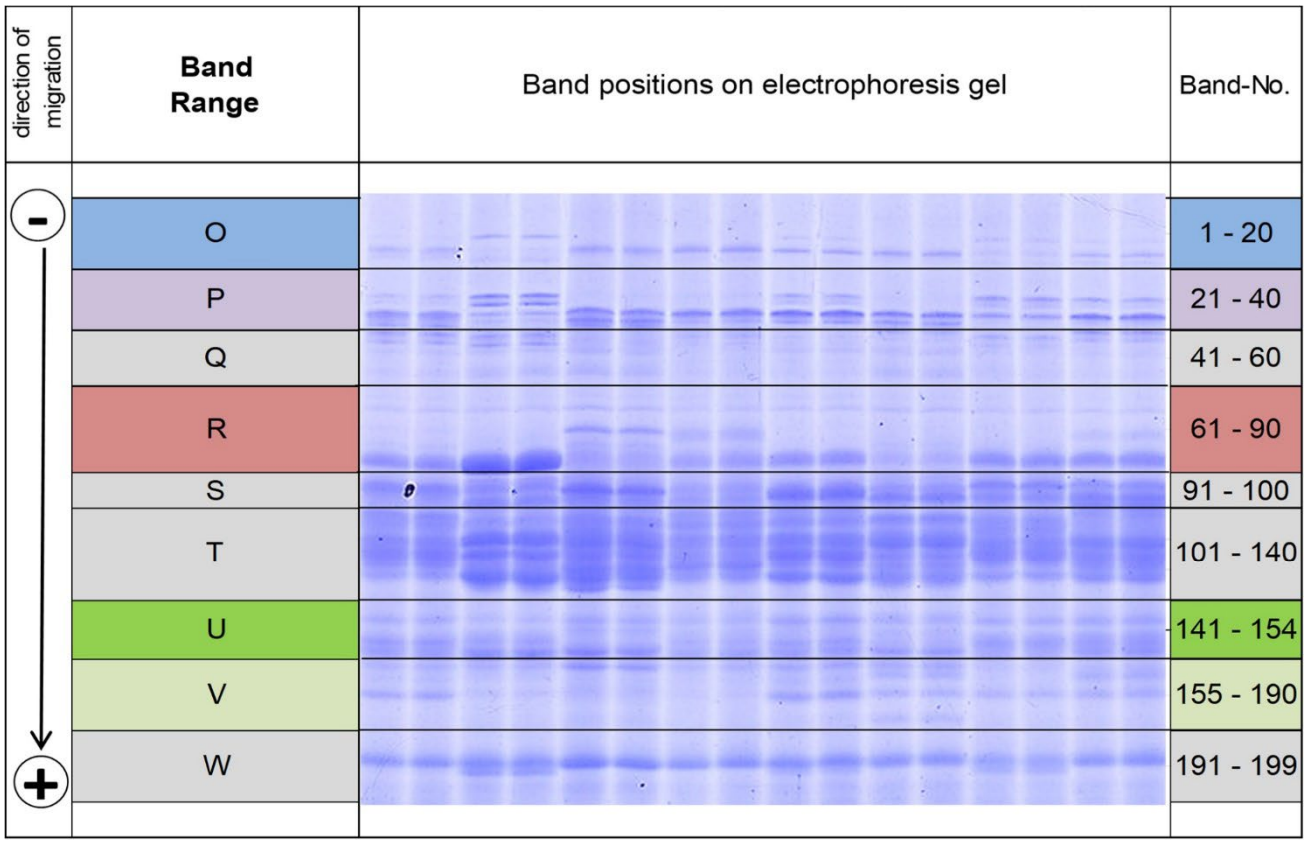
If a vertical dual slab gel instrument for example “Multigel Modell BSA” (Biometra) is used, the conditions for the electrophoresis are the following:

Component	Condition
Electrophoresis buffer:	Solution (1.3.4.2.11), fill up chamber
Voltage:	120 V (for 20 minutes), then 230 V
Current:	120 mA
Temperature:	5°C to 15°C
Running distance:	when bromophenol blue runs out at the end of the gel, proceed electrophoresis for another 40 minutes before end of run

3.5.6 Staining

2 gels from the SDS-PAGE are marked, eg by cutting the gels corner. Then the gels are transferred in a staining container filled with 300 ml staining solution (3.4.3.2) and incubated on a rocking platform shaker for 3 hours. The gels remain in the staining solution over night without shaking. For de-staining, the gels are incubated on the shaker in tap water for 2 x 30 min. Finally the gels are incubated on the shaker in 5 % glycerol solution .3.4.3.3) for 5 min. After this incubation the gels are dried between two layers of cellophane soaked in 5 % glycerol solution (3.4.3.3) at room temperature.

4 Interpretation of the Gels



Range Q, S, T and W are not analysed

Band range - O

Type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Bands	4 8 12		8 9	8 12	4 8 10	4 8	2 8	2 8 12	2 8 14	8 14	8 18	8 12 16	4 8 10 20	8 10	6 7	2 8 9	8 20	6 10	1 6 16	7 8 14	8 16 18	8 10 20	8 10 12	8 14 18	1 8 10 14	7 8 9 14	9 10 14	9 10 16
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	Lilora	Tetramax	Fornido	(none)	Eugenius	Diwan	Bellamini	Henrietta	Traffic	Hugo 1	Expert	Expert	Arsenal	Dressaro	Parcour	Phoenix	Option	Mezquita	Canberra	Ragtime	Twymax	(none)	(none)	Boccacio	Melpaula	Maiko	Resista	Tornado

Example Variety

Band Range P

Type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	22													28		26	24		26		28
	28	28	28		28					30		28	28	32	29	30	30	26	28		30
Bands	30	30	30	28	32	30		34	28	32		32	34	33	34	32	32	32	34	24	32
	36	36	36	36	36	36	36	36	30	36	36	36	36	36	36	36	36	36	36	36	36
	38	38	40	38	38	40	40	40	36	40	38	40	40	40	39	38	40	40	40	40	40
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Example Variety	Lilora	Henrietta	Rokade	Kelvin	Aut	Abosan 1	Virtuose	Bocardi	Sabor	Defender	Dasher 3	(none)	Kilrea	Indra	(none)	Melpaula	Resista	(none)	Maritim	Bargizmo	Sanova

Example Variety

Band range - R

Type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
								64							62	64	64	64				64	64		64			
		64			64		64	70				64			64	66	72	70	62	64	64	72	72		68	64	64	64
Bands	64	68	64	64	70	64	70	72	64	64	64	76	64		70	70	78	72	64	72	72	74	76	64	70	76	70	76
	70	70	66	68	76	72	78	78	72	76	76	82	80	64	78	78	80	76	70	80	76	78	80	80	78	80	74	80
	82	82	82	82	82	84	82	82	82	82	84	84	82	82	82	82	82	82	82	84	84	84	82	84	82	82	80	84
62															--				--									
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84						--					--	--								--	--	--		--				--
Example Variety	Lilora	(none)	Aberdart	Baraudi	Twymax	Cleopatra	Astonhockey	(none)	Henrietta	Dexter 1	Virtuose	Colorado	Cleancut	Traffic	Barnhem	Probat	Arusi	Maggie	Liszt 1	Eiffel	Ventoux	Alfonso	Chouss	Defender	Channi	Barsaxo	Eurosport	(none)

Example Variety

Band range - U

Type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Bands	142 148 154	144 148 154	144 150 152 154	148 150 154	148 150 152 154	142 146 148 154	142 146 154	144 150 154	144 150	142 146 150 154	142 148 152 154	142 150 152 154	142 152 154	142 150 154	146 150 154	144 148 150 154	142 148 150 154	142 144 146 154	144 148 152 154	142 144 150 154	142 144 152 154	142 146 152 154	144 146 154	142 144 146 150 154	142 144 146 148 154	142 144 146 152 154	144 145 146 148 150 154
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144		--	--					--	--							--		--	--	--	--		--	--	--	--	--	--
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154	--	--	--	--	--	--	--	--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Example Variety	Ventoux	(none)	Henrietta	Pontiac	Phoenix	(none)	Lilora	Garbor	Sabor	Poseidon	Toulouse	Mezquita	(none)	Cadix	Aberdart	Elegana	Barpasto	Denver 5	Eiffel	Vesuvius	Carillon	Mateon 1	(none)	Xanthus	Gemma	(none)	Sures	Sarwal

Example Variety

Band range - V

Type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Bands	158 162 170	162 166 168	158 164 166	158 166 176	158 176	158 162 166 176	158 162 168 178	160 166 176	156 158 160 164 166 176	156 160 166	164 168	164 166	164	156 164 170	160 166	156 162 168	158 166 168	158 168	156 164 166 176	160 166 168	158 162 166	168	158 164 170	158 162 164 166 168	156 158 170	156 166	158 166	158 160 168
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172																												
174																												
176				--	--	--		--	--										--									
178							--																					
180																												

Example Variety	Lilora	Henrietta	Garbor	Cleancut	Herbal	Akurat	(none)	Montando	(none)	Aberdart	Elegana	Traffic	(none)	Acento	Barnhem	Denver	Ragtime	Greenfair	Arusi	Birtley	Carillon	Neruda	Barmaxima	Masai	(none)	Dressano	Valerio	Ibizal

Example Variety

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The Animal and Plant Health Agency (APHA) is an executive agency of the Department for Environment, Food & Rural Affairs, and also works on behalf of the Scottish Government and Welsh Government.