



Animal &
Plant Health
Agency

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

Italian Ryegrass

Lolium multiflorum L.

December 2022

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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 Italian Ryegrass entered for Variety Listing (VL) trials and Plant Breeders' Rights (PBR).

2 Scope

2.1 These procedures apply to all varieties of Italian Ryegrass (*Lolium multiflorum* 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
The 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
BT6 9SH

Tel no	02890 548000
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
Italian Ryegrass VCU Protocol	United Kingdom Variety List Trials: Protocol and Procedures for Examining the Value for Cultivation and Use (VCU) of Italian 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 TG4/8	Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, Ryegrass (05.04.2006).
UPOV TC/33/7	Combined Over-Years Criterion for Distinctness (COYD) and Uniformity (COYU), (Revision of document TC/30/4) (09.12.1997).
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
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

Reference	Title
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.

2.2 Testing will be carried out according to these procedures. Any changes to the procedures (including new characteristics) should be agreed in advance of submitting an application, by contacting APHA.

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 website (<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).

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. 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 1st Test Cycle

Italian Ryegrass - diploid	2400 g*
Italian 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) Italian Ryegrass for seed requirements.

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. 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

- AFP number (if known)
- Breeder's reference number or name
- 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 Italian Ryegrass (*Lolium multiflorum* 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 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 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 an Italian Ryegrass seed crop in the previous five years but may be less where it has been determined the risk is negligible.

5.2 Crop 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 three 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.

5.6 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.

5.7 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.

5.8 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.

5.9 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 the sample.

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

6.5 In the third test cycle, characters, as indicated in Section D are measured on all varieties and the data analysed and, together with those from the first and second test cycles, 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, and a photographic record taken.

7 Communication with the Applicant

7.1 The Test Centre will notify the applicant or his 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 test cycle 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 three independent growing cycles although varieties may be determined DUS after two 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

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

Routine Characteristics

Code	Definition
*	A characteristic which must be examined according to UPOV Guidelines.
G	A grouping characteristic
D	A characteristic used in the variety description.

5.1 Italian Ryegrass Characteristics Routinely Recorded in DUS Tests

CPV O TP00	UPOV TG/4/8	Character	Sample source (Material examined)	Number of plants or sample size	Method of assessment and recording	States of expression	D Method Minimum difference required	U Method UPOV
	1*	Plant: ploidy	Single spaced plant test (DUS plot)	60	TQ declaration/ laboratory assessment	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 vernalisation)	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 vernalisation)	DUS plot	60	Observation	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 7=strong	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 Very tall	COYD @1%	COYU @ 0.1%

CPV O TP00	UPOV TG/4/8	Character	Sample source (Material examined)	Number of plants or sample size	Method of assessment and recording	States of expression	D Method Minimum difference required	U Method UPOV
12D		Plant: growth habit at inflorescence emergence	DUS plot	60	Computer derived (UK 11/10)	1=very erect 3=erect 5=medium 7=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%
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%

CPVO TP004/ 2	UPOV TG/4/8	Character	Sample source (Material examined)	Number of plants or sample size	Method of assessment and recording	States of expression	D Method Minimum difference required	U Method UPOV
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. These characters should only be used as a complement to confirm other morphological or physiological differences.

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	U Method UPOV UPOV
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 D	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	33	Inflorescence: length of basal spikelet plus awn	DUS plot	60	Measured	1=very small 3=small 5=medium 7=large 9=very large	COYD @1%	COYU @ 0.1%
N/A	N/A	38	Inflorescence: length of awn	DUS plot	60	Computer derived (33-35)	1=very short 3=short 5=medium 7=long 9=very long	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%

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	U Method UPOV UPOV
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%
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	10 3	Plant: seasonal width	DUS plot	60	Computer derived (UK $\sqrt{11*70}$)	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 $\sqrt{14*15}$)	1=very small 3=small 5=medium 7=large 9=very large	COYD @1%	COYU @ 0.1%
N/A	N/A	10 1	Inflorescence: total basal spikelet length	DUS plot	60	Computer derived (UK $\sqrt{24*35}$)	1=very short 3=short 5=medium 7=long 9=very long	COYD @1%	COYU @ 0.1%
N/A	N/A	10 2	Inflorescence: spikelet protuberance	DUS plot	60	Computer derived (UK 35-34)	1=very short 3=short 5=medium 7=long 9=very long	COYD @1%	COYU @ 0.1%
N/A	N/A	10 7	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	10 8	Plant: volume	DUS plot	60	Computer derived (UK $\sqrt{11*10}$)	1=very small 3=small 5=medium 7=large 9=very large	COYD @1%	COYU @ 0.1%
N/A	N/A	11 3	Inflorescence: spikelet plus awn protuberance	DUS plot	60	Computer derived (UK 33-34)	1=very small 3=small 5=medium 7=large 9=very large	COYD @1%	COYU @ 0.1%
N/A	N/A	11 7	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%

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	U Method UPOV UPOV
N/A	N/A	11 8	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	25 D	Inflorescence: awns	DUS plot	60	Observations	0=absent 1= present	For description purposes only	
N/A	N/A	12 4D	Seedling: fluorescence	Laboratory test	60	Observations	0=absent 1= present	For description purposes only	

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 TP004/ 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	U Method UPOV
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	12 1	Seedling: tiller Number	Glasshouse trial	180	Measured	1=none 3=small 5=medium 7=large 9=very large	ANOVA (t-test) @ 1% ¹	Ftest @ 1% ²
N/A	N/A	12 2	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	Ftest @ 1% ²
N/A	N/A	12 3	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	Ftest @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.

5.5 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 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-tetraploid varieties) 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 number of seed requested will be 1500g for diploid varieties and 2000g for tetraploid varieties (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 Italian Ryegrass entered for Variety Listing trials 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 for determinations of DUS.

3 Responsibilities

3.1 The Test Centre is responsible for liaising with the applicant to produce a proposed procedure for the conduct of new tests. This procedure must ensure that Distinctness, Uniformity and Stability 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 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 5.1, 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 distinctness, uniformity and stability of varieties of Italian Ryegrass.

2 Scope

2.1 These procedures apply to all varieties of Italian Ryegrass (*Lolium multiflorum* 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 report 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.



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