Animal &
Plant Health
Agency

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

Brussels Sprouts

Brassica oleracea L. var. *gemmifera* DC.

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 and maintenance of reference stocks of varieties of Brussels Sprout entered for Variety Listing (VL) Trials and Plant Breeders' Rights (PBR).

2 Scope

2.1 These procedures apply to all varieties of Brussels Sprout (*Brassica oleracea* L. var. *gemmifera* DC.). 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; the 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 the:

Vegetable DUS Test Centre

SASA

Roddinglaw Road

Edinburgh Tel No 0131244 8890

EH12 9FJ Fax No 0131244 8940

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
UPOV TG/54/7 Rev	Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, Brussels Sprout (*Brassica oleracea* L. var *gemmifera* DC.). 31.03.2004 + 16.03.2016.
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. 09.04.2002.
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.
GB and NI Variety Lists	The Seeds (National Lists of Varieties) Regulations 2001 (as amended) and The Seeds (Variety Lists) Regulations (Northern Ireland) 2020
Plant Varieties Act 1997	Plant Breeders' Rights Regulations 1998 and Plant Varieties Act 1997
Plant Breeders' Rights 2019	The Plant Breeders' Rights (Amendment etc.) (EU Exit) Regulations 2019 as amended by The Animal Health, Invasive Alien Species, Plant Breeders' Rights and Seeds (Amendment etc.) (EU Exit) Regulations 2019 and The Plant Breeder 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 website at https://www.gov.uk/national-lists-of-agricultural-and-vegetable-crops

4.3 Applicants should 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 iis 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

2,000 or 6,000 seeds

7.2 2nd Test Cycle

4,000 seeds if 2,000 seeds were provided in 1st test cycle.

No seed if 6,000 seeds were provided in 1st test cycle.

Where a second sample has been provided, it will be authenticated against the original submission. An additional charge will be applied.

7.3 Shortfall in Seed Quantities

Where insufficient seed is available in the first instance a further stock must 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 with the following information:

- Applicant
- AFP number (if known)
- Breeder's reference number or name
- Quantity of seed.
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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 Brussels Sprout (*Brassica oleracea* L. var. *gemmifera* DC.).

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 DUS Test Centre.

5 Design of Tests

5.1 The DUS Test Centre is responsible for selecting a suitable site which should be on ground that has normally not had a Cruciferous crop in the previous five years but may be less where it has been determined that the risk is negligible.

5.2 Field husbandry should follow best practice for all operations and particularly as regards cultivation, drilling, fertiliser, transplanting and spray application, use of irrigation and control of weeds, 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 TQ the candidate variety may be grown in plots and compared with varieties which are in the same classification for the following characters:

Characteristics that could be used for grouping:

Plant: height (characteristic 1)

Leaf blade: colour (characteristic 5)

Leaf blade: intensity of colour (characteristic 6)

Leaf blade: cupping (characteristic 8)

Time of harvest maturity (characteristic 19)

Male sterility (characteristic 21)

Plant: tendency to form a head (characteristic 2)

Leaf blade: blistering (characteristic 8)

5.5 Varieties known to be clearly distinct from the candidate on any other discontinuous or continuous characteristic listed in Section D 5.1 may be excluded from the trial. See Section F for further information on additional characters.

5.6 The tests are carried out using a grouped design, with a plot of each candidate variety present in each replicate as follows:

Number of replications	2
Number of rows per plot	2
Spacing between plot rows	0.6m
Plot length	10 m
Number of plants per replicate	at least 32
Plant spacing	0.6m (approx.)

Groups are randomised and varieties are randomised within groups.

5.7 Seed is sown in the glasshouse between mid-April and early May and transplanted into the field between late May and early June according to a plan produced by the Test Centre. Varieties are coded by the Test Centre.

5.8 Any candidate with distinctness problems in the first test cycle may be grown side by side with their close controls in the second or third test cycles.

5.9 Recordings are taken on each trial from approximately 16 weeks after sowing until harvest maturity stage. Characters recorded are listed in Section D.

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 noted but excluded from analysis.

6.3 In the first recording cycle, characters, as indicated in Section D 5.2, are recorded on all candidates and their close controls. The data for measured characters are analysed and used to determine the most similar reference varieties and assess uniformity of the candidate. (For details see Section G).

6.4 In the second recording year, characters, as indicated in Section D5.2, are recorded on all candidates and their close controls. The data for measured characters are analysed and, together with those from the first recording cycle, used to determine the most similar reference varieties and assess the uniformity of the candidate. (For details see Section G).

6.5 If a third test cycle of test is necessary, characters, as indicated in Section D 5.2, are recorded on all candidates and their close controls. The data for measured characters are analysed and, together with those from the first and second cycles, are used to determine the most similar reference varieties and assess the uniformity of the candidate. (For details see Section G).

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

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 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, methods of observation and standards required for DUS assessment.

3 Responsibilities

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

4 Organisation

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

5 DUS Characteristics to be Assessed

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

Type of observation of characteristics:

MG Single measurement of a group of plants of parts of plants

MS Measurement of a number of individual plants or parts of plants

VG Visual assessment by a single observation of a group of plants or parts of plants

VS Visual assessment by observation of individual plants or parts of plants

5.2 Brussels Sprout Characteristics Routinely Recorded in DUS Tests

UPOV TG/54/7 2016	UK code	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 and Standard applied
*1G QN VG/MG	*DG	**Plant: height**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score or single measurement per plot	3 = short 5 = medium 7 = tall	Clear visual difference or 2 states difference or COYD @ 5%	Visual Assessment or for measured samples COYU @ 0.1%
2 QN VG	*2G	**Plant: tendency to form a head**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1 = absent or very weak 3 = weak 5 = medium 7 = strong 9 = very strong	Clear visual difference or 2 states	Visual Assessment
*3 QN VG	*3	**Leaf blade: size**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 = small 5 = medium 7 = large	Clear visual difference or 2 states	Visual Assessment
4 QN VG	19	**Leaf blade: length**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 = short 5 = medium 7 = long	Clear visual difference or 2 states	Visual Assessment
*5G PQ VG	*4G	**Leaf blade: colour**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1 = green 2 = blue green 3 = purple	Clear visual difference or 1 state	Visual Assessment

UPOV TG/54/7 2016	UK code	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 and Standard applied
*6G QN VG	*5G	**Leaf blade: intensity of colour**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 = light 5 = medium 7 = dark	Clear visual difference or 2 states	Visual Assessment
7 QN VG	*6	**Leaf blade: waxiness**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 = weak 5 = medium 7 = strong	Clear visual difference or 2 states	Visual Assessment
*8G QN VG	*7G	**Leaf blade: cupping**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 = moderately convex 5 = plane 7 = moderately concave 9 = strongly concave	Clear visual difference or 2 states	Visual Assessment
9 QN VG	*8G	**Leaf blade: blistering**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 = weak 5 = medium 7 = strong	Clear visual difference or 2 states	Visual Assessment
10 QL VG	9	**Leaf blade: reflexing of margin**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1 = absent 9 = present	Clear visual difference or 1 state	Visual Assessment
*11 QN VG	*10	**Petiole: attitude**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 =semi erect 5=horizontal 7=semi pendulous	Clear visual difference or 2 states	Visual Assessment
12 QN VG	11	**Petiole: length compared to blade**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3= moderately shorter 5 = equal 7 = moderately longer	Clear visual difference or 2 states	Visual Assessment
13 QN VG	20	**Petiole: anthocyanin coloration**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1 = absent or very weak 3 = weak 5 = medium 7 = strong 9 = very strong	Clear visual difference or 2 states	Visual Assessment

UPOV TG/54/7 2016	UK code	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 and Standard applied
14 PQ VG	14	**Sprout: shape in longitudinal section**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1 = narrow obovate 2 = obovate 3 = broad obovate 4 = circular	Clear visual difference or 2 states	Visual Assessment

UPOV TG/54/7 2016	UK code	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 and Standard applied
15 PQ VG	*15	**Sprout: colour**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1 = green 2 = blue green 3 = purple	Clear visual difference or 1 state	Visual Assessment
16 QN VG	16	**Sprout: intensity of colour**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 = light 5 = medium 7 = dark	Clear visual difference or 2 states	Visual Assessment
17 QN VG	21	**Sprout: density at harvest maturity**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 = loose 5 =medium 7 = dense	Clear visual difference or 2 states	Visual Assessment
18 QN VG	*17	**Stem: spacing of sprouts**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3 = narrow 5 = medium 7 = wide	Clear visual difference or 2 states	Visual Assessment
*19G QN VG	*18G	**Time of harvest maturity**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score or single value (date) per plot	1 = very early 3 = early 5 = medium 7 = late 9 = very late	Clear visual difference or 2 states or COYD @ 5%	Visual Assessment or for measured samples COYU @ 0.1%
20 QN VG	22	**Stem: profile of sprout column**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1 = conical 2 = conical to cylindrical 3 = cylindrical	Clear visual difference or 2 states	Visual Assessment
21G QL VS	23G	**Male sterility**	20 single plants	At least 20 plants in total	Visual observation on single plants in 2 replicates	1 = absent 9 = present	Clear visual difference or 1 state	Visual Assessment

5.3 Previously Approved Characteristics Not Routinely Recorded in DUS Tests

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, Plant Varieties and Seeds.

UPOV TG/54/7 2016	UK code	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 and Standard applied
N/A	24 QL VG	**Hypocotyl: anthocyanin pigment**	Seedlings raised in module trays	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=absent 9=present	Clear visual difference or 1 state	Visual Assessment
N/A	25 PQ VG	**Cotyledon: colour**	Seedlings raised in module trays	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=yellow green 2=light green 3=dark green 4=blue-green 5=violet green	Clear visual difference or 1 state	Visual Assessment
N/A	26 QN VG	**Plant: width**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3=narrow 5=medium 7=wide	Clear visual difference or 2 states	Visual Assessment
N/A	27 QN VG	**Plant: leaf abscission**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3=early 5=medium 7=late	Clear visual difference or 2 states	Visual Assessment
N/A	28 QN VG	**Leaf blade: anthocyanin colouration**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=absent or very weak 3=weak 5=medium 7=strong 9=very strong	Clear visual difference or 2 states	Visual Assessment
N/A	29 QL VG	**Leaf blade: pigment on mid-vein**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=absent 9=present	Clear visual difference or 1 state	Visual Assessment

UPOV TG/54/7 2016	UK code	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 and Standard applied
N/A	30 QL VG	**Leaf blade: pigment at lamina tip**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=absent 9=present	Clear visual difference or 1 state	Visual Assessment
N/A	31 QN VG	**Leaf blade: crumpling**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=absent 3=slight 5=medium 7=much	Clear visual difference or 2 states	Visual Assessment
N/A	32 QN VG	**Leaf blade: twisting**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=absent 3=slight 5=medium 7=much	Clear visual difference or 2 states	Visual Assessment
N/A	12 QN VG	**Leaf blade: width**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3=narrow 5=medium 7=broad	Clear visual difference or 2 states	Visual Assessment
N/A	33 QL VG	**Leaf blade: pigment on mid-vein**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=absent 9=present	Clear visual difference or 1 state	Visual Assessment
N/A	34 QL VG	**Leaf blade: pigment at lamina tip**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=absent 9=present	Clear visual difference or 1 state	Visual Assessment
N/A	35 PQ VG	**Leaf blade: shape**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	1=narrow elliptic 2=elliptic 3=broad elliptic 4=circular 5=transverse elliptic	Clear visual difference or 2 states	Visual Assessment

UPOV TG/54/7 2016	UK code	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 UPOV TC/33/7	U Method UPOV TC/33/7
N/A	36 QN VG	**Petiole: length**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3=short 5=medium 7=long	Clear visual difference or 2 states	Visual Assessment
N/A	37 QN VG	**Petiole: width at mid point**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3=narrow 5=medium 7=wide	Clear visual difference or 2 states	Visual Assessment
N/A	38 QN MS	**Sprout: length**	DUS plot	At least 20 plants in total from 2 replicates	Single plant measurements	3=short 5=medium 7=long	COYD @ 5%	Visual Assessment or for measured samples COYU at 0.1%
N/A	39 QN MS	**Sprout: width**	DUS plot	At least 20 plants in total from 2 replicates	Single plant measurements	3=narrow 5=medium 7=wide	COYD @ 5%	Visual Assessment or for measured samples COYU at 0.1%
N/A	13 QN VG	**Sprout: size**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3=small 5=medium 7=large	Clear visual difference or 2 states	Visual Assessment
N/A	40 QN VG	**Sprout column: stem length**	DUS plot	At least 40 plants in total from 2 replicates	Visual observation or visual score	3=short 5=medium 7=tall	Clear visual difference or 2 states	Visual Assessment

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

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.

3 Responsibilities

3.1 The Test Centre is responsible for conducting these procedures.

4 Procedures for Reference Seed Stock Maintenance

4.1 The seed sample submitted with the successful or pending application is considered to be the definitive stock of the variety. Subject to meeting the required certification standards a small portion of the seed is sown for observation and measurement. The remainder is stored under controlled and monitored storage 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 have low quantity, a request will be made to the maintainer asking for a replacement stock of the variety. This replacement stock must be authenticated, by comparing plots established from the replacement seed with that of the definitive seed, over a maximum of two recording cycles.

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 test cycles. Plots must be examined through all stages of growth and development. If the new seed sample cannot be visually distinguished from the existing definitive seed it will be accepted as representing the variety. It will then be considered as the definitive seed and substituted for the existing seed in the

reference collection. These procedures may be modified, where, in the opinion of the technical officer, differences are the result of environmental or cultural factors.

4.4 In the event of the replacement sample not meeting the required acceptance standards set out in 4.3, an additional replacement sample will be 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 standards, APHA will be informed and the variety will be deleted from the reference collection and the Variety Lists will be reviewed.

5 Procedures for The Inclusion of New Common Knowledge Varieties into the Reference Collection

5.1 When a new variety enters into common knowledge such that it must be included in the reference collection if seed is available. A request for seed will be sent by the Test Centre to the maintainer of the variety and an official description will be requested from the Testing Authority which registered the variety. If an official description is provided, seed received will be assumed to be definitive if the seed conforms to the official description. Small differences in the expression of quantitative characters are likely to be the result of recording in a different environment, and will be considered as conforming to the description. If no official description is available, seed will be assumed to be definitive.

5.2 If the seed does not conform to the official description, a request for definitive seed will be sent to the Testing Authority that added the variety to its Variety List or granted Plant Breeders' Rights. This seed will then be used to validate the sample of seed from the maintainer. The standards for this validation will be as for authentication of replacement seed (see E4).

6 Release of Reference Samples for Authorised Purposes

6.1 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.

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

Section F - Procedures for Assessment of New Additional DUS Characters

1 Purpose

1.1 This section sets out the procedures for assessment of new additional DUS characters for varieties of Brussels Sprout entered for Variety Listing and/or Plant Breeders' Rights trials.

2 Scope

2.1 These procedures apply to applications where additional DUS characteristics which have not been previously approved by the NLSC are requested for use in the examination of DUS.

3 Responsibilities

3.1 The Test Centre is responsible for liaising with the applicant to produce a proposed procedure for the conduct of new tests. This procedure must ensure that Distinctness, Uniformity and Stability 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 5.2 or 5.3 as appropriate.

Section G - Procedures for DUS Decisions

1 Purpose

1.1 This section sets out the procedures used to assess distinctness, uniformity and stability of varieties of Brussels Sprout.

2 Scope

2.1 These procedures apply to all varieties of Brussels Sprout (*Brassica oleracea* L. var. *gemmifera* DC.) 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 UPOV guidelines.

4 Reference Varieties

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

5 Distinctness

5.1 Distinctness is normally assessed in two independent test cycles, but a candidate variety could be considered distinct after one test cycle if there are no other similar varieties. A third independent test cycle may be undertaken if distinctness is not established after two test cycles.

5.2 In accordance with associated document UPOV TG/1/3 varieties can be considered distinct where they have a different expression in a grouping character.

5.3 The distinctness standard applied for qualitative characters is a difference of one state, unless otherwise indicated in Section D. For pseudo-qualitative characters the distinctness standard is a difference of 1, 2 or 3 states depending on the characteristic.

5.4 If a candidate is clearly different in a visually observed quantitative character, it is considered to be distinct without the need for a repeated observation.

5.5 Where varieties are grown in close proximity under the same conditions, and a direct comparison can be made, a candidate is considered to be distinct if a clear visual difference is observed in a quantitative character.

5.6 Where varieties are not grown in close proximity, a candidate is considered to be distinct if a difference of two states (see table in section D 5.1 and D5.2) is recorded in a visually observed quantitative character.

5.7 The standard for measured or counted quantitative characters, is, at least, a 5% (P=0.05) significant difference in one character over two or three growing cycles in a Combined Over Years Distinctness (COYD) analysis. Please see associated document UPOV TGP/8/1 for details.

5.8 Where COYD cannot be applied, alternative methods should be considered.

5.8.1 When the number of varieties grown does not provide sufficient degrees of freedom for use of the standard COYD analysis, alternative methods should be adopted. If there is sufficient historical data (at least 5 years and sufficient degrees of freedom) then the long term LSD is applied. This LSD is calculated using up to 10 years of the most recent data. If there is insufficient historical data, the 2 x 1% method should be used.

5.8.2 Where the candidate has a full complement of data for two test cycles, but there is only data for control varieties for one test cycle, the use of FITC (Fitted Constant program in DUST) may be applied. This situation may arise due to the loss of plant material within plots in any one year or where suitable control varieties were not grown in both test cycles. The standard applied for Distinctness in such cases is P=0.01.

6 Uniformity

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

Uniformity based on the assessment of ‘Off-types’

6.2 The assessment of Off-types is undertaken in both test cycles and the total number of ‘off-types’ combined should not exceed the number allowed using the population standards.

Off-type plants in the glasshouse or field are identified and marked for exclusion from recording.

6.3 For cross-pollinated varieties relative uniformity standards are applied; the total number of off-type plants recorded in the test should not exceed that of similar varieties.

6.4 In single cross hybrids and self-pollinated varieties (inbred lines) the total number of off-types plants should not exceed that indicated in UPOV TGP/8/1 using a population standard of 1% and a 95% acceptance probability. In a population of 60 plants, 2 off-types are allowed.

6.5 Where the number of off-types in the first test cycle exceeds 2 but is less than 4, the applicant may submit a new seed sample (Resubmission) in the second test cycle with the aim of meeting the off-type standard. Distinctness will be assessed on data from the original seed submitted in the first test cycle and on data from the resubmitted seed in the second test cycle. The resubmitted seed will be authenticated against the original seed in side-by-side plots.

6.6 In addition, the number of aneuploid or inbred plants allowed in F1 hybrids should not exceed the numbers indicated in UPOV TGP/8/1 for a 3% population standard and a 95% acceptance probability.

6.7 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 described.

Uniformity based on the assessment of general variation where no measurements are recorded:

6.8 Uniformity of continuous variation (quantitative characters) is visually assessed according to the following scale:

Score 1-5 unacceptable (1 is worst)

Score 6-9 acceptable (9 is best)

A candidate with a visual uniformity score of 6 or more is satisfactory.

Uniformity based on the assessment of general variation where measurements are recorded:

6.9 Provided a variety meets the off-type standard, it can be considered sufficiently uniform after two, or three, test cycles if, for all measured characters necessary 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 (see document UPOV TGP/8/1). In all cases an examination of data from individual test cycles is carried out to investigate the uniformity problem indicated by the COYU result. Decisions on whether any outlier plants (off-types) identified by data analysis should be excluded from the calculation of variety means and standard deviations, should be taken by the Test Centre.

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 the 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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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.