UNITED KINGDOM NATIONAL LIST/ PLANT BREEDERS RIGHTS TECHNICAL PROTOCOL FOR THE OFFICIAL EXAMINATION OF DISTINCTNESS, UNIFORMITY AND STABILITY (DUS)

BRUSSELS SPROUT

(*Brassica oleracea* L.*)*

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 National List (NL) and Plant Breeders' Rights (PBR) tests.

2 SCOPE

2.1 These procedures apply to all varieties of Brussels Sprout. Special procedures and responsibilities for Genetically Modified (GM) varieties are set out in Sections A5 and A6.

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

3 RESPONSIBILITIES

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

3.2 They are supervised, on behalf of the National Authorities, by officials of the Testing Authorities, that is the Animal and Plant Health Agency (APHA), the Scottish Government Agriculture and Rural Development Division (SGARD), 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 their approval. Requests and suggestions for amendment of the protocol should be put in writing to APHA, Plant Varieties and Seeds, either directly or via the Test Centre.

3.4 The procedures are administered by:

Plant Varieties and Seeds
Animal and Plant Health Agency
Eastbrook
Shaftesbury Road
Cambridge Tel No: 02080 265993
CB2 8DR Fax No: 02084 152504

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
Science and Advice for Scottish Agriculture (SASA)
Roddinglaw Road
Edinburgh Tel No 0131-244 8853
EH12 9FJ Fax No 0131-244 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, Plant Varieties and Seeds. Where this protocol uses the word “should” for any action this is the method to be followed unless there are clear reasons not to do so which can be justified by the Test Centre as technically sound.

5 RESPONSIBILITY FOR GM RELEASES

5.1 GM Release Consent Holders are responsible for GM releases. All parties involved in DUS work operating under a GM Release Consent must adhere to the instructions of the Release Consent Holder where necessary, to comply with the relevant consent conditions. Where DUS protocol non-compliance occurs, this must be reported to the consent holder and the Test Centre who will notify APHA, Plant Varieties and Seeds.

6 PROCEDURES FOR GM VARIETIES

6.1 Applicants intending to enter GM candidates must consult APHA, Plant Varieties and Seeds, 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, Plant Varieties and Seeds has given the specific clearances.

7 ASSOCIATED DOCUMENTS

7.1 The following documents are associated with this protocol:

Reference	Title
CPVO-TP/054/2 Rev	Protocol for Distinctness, Uniformity and Stability tests of *Brassica oleracea* L. var*. gemmifera* Zenker. 15.03.2017.
UPOV TG/54/7 Rev	Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability, Brussels Sprout (*Brassica oleracea* L. var *gemmifera* DC.). 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/8/3	Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability. 28.10.2016.
UPOV TGP/9/2	Examining Distinctness. 29.10.2015.
UPOV TGP/10/1	Examining Uniformity. 30.10.2008.
Commission Directives	Commission Directive of 2003/91/EC, as amended, setting out implementing measures for the purposes of Article 7 of Council Directive 2002/55/EC (13th June 2002) as regards the characteristics to be covered as a minimum by the examination and the minimum conditions for examining certain varieties of vegetable species. [Brussels Sprout]
Council Regulation (EC) No. 2100/94	Council Regulation (EC) No. 2100/94 of 27th July on Community Plant Variety Rights.

SECTION B - APPLICATION REQUIREMENTS

1 PURPOSE

1.1 The purpose of this section is to identify the specific requirements for National List and Plant Breeders' Rights applications.

2 SCOPE

2.1 These procedures apply to all applications.

3 RESPONSIBILITIES

3.1 The applicants are responsible for ensuring that these procedures are complied with.

4 RECEIPT OF APPLICATIONS

4.1 The latest date for receipt of applications for acceptance of a variety onto the National List or for Plant Breeders' Rights is 31st January, which is set administratively by APHA, Plant Varieties and Seeds. Applications received after this date may be considered for inclusion in the current year's tests and trials on a case by case basis.

4.2 The procedures for the submission of National List and Plant Breeders' Rights applications, technical questionnaires (TQ) and for payment of administration fees are set out on the APHA web site at https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/714974/pbr-fees.pd.

4.3 Applicants should notify APHA, Plant Varieties and Seeds of special DUS characteristics which may require additional examinations. These claims should, in addition, be noted in the TQ accompanying the application.

5 RECEIPT OF SEED

5.1 The latest date for receipt of seed is 28th February and is set administratively by APHA, Plant Varieties and Seeds. Seed submissions received after this date will normally be refused. Instructions for the delivery of seed will be made available to applicants by APHA, Plant Varieties and Seeds.

6 SEED QUALITY REQUIREMENTS

6.1 The seed must satisfy the quality requirements for Basic Seed as laid down in Schedule 4 Part II of the Vegetable Seeds Regulations (England) 2002 SI.3175/2002, as amended, and equivalent regulations made by 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.

8 LABELLING REQUIREMENTS, INCLUDING PROVISIONS FOR GM VARIETIES

8.1 Applicants **must** clearly label their seed with the following information:

- Applicant
- Breeder's reference number or name
- Quantity of seed.
- Whether it is a parental line

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

SECTION C – GROWING TEST PROCEDURES

1 PURPOSE

1.1 The purpose of this section is to provide details of the procedures used in the growing tests for DUS analysis.

2 SCOPE

2.1 These procedures apply to all varieties of Brussels Sprout.

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 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 the risk is negligible.

5.2 Field husbandry should follow best local practice for all operations and particularly as regards cultivation, drilling, fertiliser, transplanting and spray application, use of irrigation and control of pests and diseases.

5.3 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:

CPVO grouping 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 18)
Male sterility (characteristic 20)

Additional grouping characters used in the UK:

Plant: tendency to form a head
Leaf blade: blistering

5.4 Varieties known to be clearly different from the candidate on any other discontinuous or continuous characteristic may be excluded from the trial. If this exclusion is based on a characteristic which is not listed in Section D 5.1, approval by the NLSC and CPVO must be sought. See Section F for further information on additional characters.

5.5 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	0.9m
Plot length	10 m
Number of plants per replicate	at least 30
Hence, number of plants per variety	at least 60
Plant spacing	0.6m (approx)

Groups are randomised and varieties are randomised within groups.

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

5.7 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.

5.8 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 the sample.

6.3 In the first recording cycle, characters, as indicated in Section D 5.1, 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 D5.1, 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 5.1, 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).

6.6 If the Test Centre notices unusual or novel characters in a candidate, a note may 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 his agent of any DUS problems at the earliest practical opportunity. All such notifications must be copied to APHA, Plant Varieties and Seeds. In the case of tests for foreign DUS authorities, notifications must be copied to the test authority and to APHA, Plant Varieties and Seeds. In the case of European applications, notifications must be copied to CPVO and APHA, Plant Varieties and Seeds.

7.2 If confidentiality considerations allow, the applicant should be informed which variety is similar to his own and be invited to submit any information which may help to distinguish them.

7.3 If DUS problems arise, applicants will be invited to visit the DUS tests by arrangement so that the material can be examined and discussions held with the Test Centre.

7.4 After each recording cycle the results are summarised and reported by the Test Centre to, APHA, Plant Varieties and Seeds, who will inform the applicant, foreign test authorities or the CPVO as appropriate.

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.

4 ORGANISATION

4.1 The minimum duration of tests to assess characteristics is normally two independent growing cycles. Shorter periods may be applied for the assessment of additional characteristics. Additional growing cycles may be approved by the UK National List and Seeds Committee (NLSC).

5 DUS CHARACTERISTICS TO BE ASSESSED

5.1 Routine Characteristics

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

Legend:

Key to abbreviations used with character number

Types of expression of characteristics:
QL - Qualitative characteristic
QN - Quantitative characteristic
PQ - Pseudo-qualitative characteristic

Types of observation of characteristics:
MG - Single measurement of a group of plants or parts of plants
MS - Measurement of a number of individual plants or parts of plants
VG - Visual assessment by a single observation of a group of plants or parts of plants
VS - Visual assessment by observation of individual plants or parts of plants

G denotes a grouping characteristic.
D denotes a characteristic used in the variety description.

A.1.1.1.

B.1.1.1. Note: For the CPVO numbered characteristics, all characteristics in the list are compulsory; notwithstanding, in the case of disease resistance characteristics, only those resistances marked with an asterisk (*) in the CPVO column are compulsory.

C.1.1.1.

BRUSSELS SPROUT CHARACTERISTICS ROUTINELY RECORDED IN DUS TESTS

Character Number									
CPVO TP/54/2 2017	**UPOV TG/54/7 2016**	**UK**	**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**
1DG QN VG/MG	*1DG QN VG/MG	*1DG	**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% for both 2 and 3 year tests	Off-type standard and Uniformity score >5
2D QN VG	2D QN VG	*2DG	**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	Off-type standard and Uniformity score >5
3D QN VG	*3D QN VG	*3D	**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	Off-type standard and Uniformity score >5
4D QN VG	4D QN VG	19D	**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	Off-type standard and Uniformity score >5
5DG PQ VG	*5DG PQ VG	*4DG	**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	Off-type standard
6DG QN VG	*6DG QN VG	*5DG	**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	Off-type standard and Uniformity score >5
7D QN VG	7D QN VG	*6D	**<u>Leaf blade: waxiness</u>**	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	Off-type standard and Uniformity score >5
8DG QN VG	*8DG QN VG	*7DG	**<u>Leaf blade: cupping</u>**	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	Off-type standard and Uniformity score >5
9D QN VG	9D QN VG	*8DG	**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	Off-type standard and Uniformity score >5

CPVO TP/54/2 2017	UPOV TG/54/7 2016	UK	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
10D QN VG	*11D QN VG	*10 D	**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	Off-type standard and Uniformity score >5
11D QN VG	12D QN VG	11D	**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	Off-type standard and Uniformity score >5
12D QN VG	13D QN VG	20D	**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	Off-type standard and Uniformity score >5
13D PQ VG	14D PQ VG	14D	**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	Off-type standard and Uniformity score >5
14D PQ VG	15D PQ VG	*15 D	**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	Off-type standard
15D QN VG	16D QN VG	16D	**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	Off-type standard and Uniformity score >5
16D QN VG	17D QN VG	21D	**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	Off-type standard and Uniformity score >5
17D QN VG	18D QN VG	*17 D	**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	Off-type standard and Uniformity score >5
18DG QN VG/MG	*19DG QN VG	*18 DG	**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% for both 2 and 3 year tests	Off-type standard and Uniformity score >5
19D QN VG	20D QN VG	22D	**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	Off-type standard and Uniformity score >5

CPVO TP/54/2 2017	UPOV TG/54/7 2016	UK	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
20DG QL VS	21DG QL VS	23DG	**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	Off-type standard

5.2 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.

Character Number									
CPVO TP/54/2 2017	UPOV TG/54/7 2016	UK	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
		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	Off-type standard
		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	Off-type standard
		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	Off-type standard and Uniformity score >5
		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	Off-type standard and Uniformity score >5
		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	Off-type standard and Uniformity score >5
		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	Off-type standard

CPVO TP/54/2 2017	UPOV TG/54/7 2016	UK	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
		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	Off-type standard
		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	Off-type standard and Uniformity score >5
		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	Off-type standard and Uniformity score >5
	10D QN VG	09 QL VG	**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	Off-type standard and Uniformity score >5
		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	Off-type standard and Uniformity score >5
		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	Off-type standard
		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	Off-type standard
		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	Off-type standard
		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	Off-type standard and Uniformity score >5
		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	Off-type standard and Uniformity score >5

CPVO TP/54/2 2017	UPOV TG/54/7 2016	UK	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
		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% for both 2 and 3 year tests	Off-type standard and COYU at 0.1% for both 2 and 3 year tests
		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% for both 2 and 3 year tests	Off-type standard and COYU at 0.1% for both 2 and 3 year tests
		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	Off-type standard and Uniformity score >5
		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	Off-type standard and Uniformity score >5

5.3 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 the authentication of replacement reference seed.

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 quality 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 If the replacement seed sample cannot be visually distinguished from the definitive reference stock, it will be accepted as representing the variety. If there are visual differences, the new sample will be recorded, and will be accepted as representing the variety if there are no significant (P=0.05) differences in the first recording cycle, or no significant (P=0.05) differences over two recording cycles in a COYD analysis (see associated document UPOV TGP/8/1 for details). It may then be accepted as definitive and substituted for the existing definitive stock 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 A replacement sample or an additional replacement sample will be considered sufficiently uniform after one recording cycle, if the level of off-types is the same or less than the number at 1% population standard and 95% acceptance probability, and the standard deviations of the measured characters are not significantly greater at the 0.1% (P=0.001) significance level than that of the mean standard deviations of the control varieties. Over 2 years the additional replacement sample will be considered sufficiently uniform if the Combined Over Years Uniformity (COYU) is not significantly greater at the 0.1% (P=0.001) significance level than that of the reference varieties. These procedures may be modified, where, in the opinion of the technical officer, differences are the result of environmental or cultural factors.

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.

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 National 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, EU and UPOV DUS Testing Authorities and UK, EU 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 the NLSC.

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 National List and Plant Breeders' Rights tests.

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 DUS testing.

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 will be assessed.

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

4 REFERENCE VARIETIES

4.1 The reference varieties will include only those varieties from which the candidate variety is not distinct, as well as other 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 by the DUS Centre to the NLSC to consider the feasibility of setting up a test acceptable to the UK Authorities. The applicant will be advised by APHA, Plant Varieties and Seeds of arrangements and costs.

5.2 The NLSC will consider the results of the commissioned test or trial when reaching its recommendation on the granting of Plant Breeders' Rights and/or National Listing.

5.3 Where the test for a character is approved by the NLSC it should be subsequently listed in Section D5.1 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 Brussels Sprout.

2 SCOPE

2.1 These procedures apply to all varieties of Brussels Sprout entered for UK National List and Plant Breeders' Rights tests and those being tested for the CPVO or for other 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 CPVO Protocols.

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 the first test cycle.

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

6.4 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.

6.5 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.

6.6 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.

6.7 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.

6.8 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:

6.9 Uniformity of continuous variation (quantitative characters) is assessed visually 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:

6.10 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, Plant Varieties and Seeds by the specified date. This report will specify all non-routine characteristics used for establishing distinctness.

8.2 The final DUS report, including the full variety description, will be submitted to APHA, Plant Varieties and Seeds by the specified date. The characteristics to be used in the description are identified in Section D.

APPENDIX 1

REFERENCE COLLECTION VARIETIES

1 NATIONAL LISTING

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

D.1.1.1. 1.2 All other candidate varieties already in DUS test in the UK, or entering testing at the same time as the candidate, including those being tested for other Member States.

E.1.1.1. 1.3 All varieties on the UK National List and varieties on the EC Common Catalogue.

F.1.1.1. 1.4 Varieties nominated by the authorities concerned where tests are done for other Member States.

G.1.1.1. 1.5 Any varieties nominated by the applicant as being comparable i.e. known to be similar.

H.1.1.1. 1.6 Any other varieties considered to be comparable i.e. known to be similar by the appropriate DUS Test Centre.

2 PLANT BREEDERS RIGHTS

2.1 The DUS reference collection, for PBR purposes, for any given category of plant variety comprises the following at the time when the application for the candidate is made.

I.1.1.1. 2.2 All other candidate varieties already in DUS tests in the UK, or entering DUS testing at the same time as the candidate, including those being tested for other Member States or the Community Plant Variety Office (CPVO).

J.1.1.1. 2.3 Varieties protected in the UK, EC or in a UPOV Member State, which are known to be similar to the candidate variety.

K.1.1.1. 2.4 Other available comparable varieties in common knowledge.