

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

BROCCOLI/CALABRESE

(Brassica oleracea L. Broccoli/Calabrese)

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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 and maintenance of reference stocks of varieties of Broccoli/Calabrese entered for National List (NL) and Plant Breeders' Rights (PBR) tests.

2 SCOPE

- 2.1 These procedures apply to all varieties of Broccoli/Calabrese (*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.[including *Brassica oleracea* L. convar.*botrytis* (L.) Alef. var. *italica*]). 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 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 Secretary of State for Northern Ireland (the National Authorities).
- 3.2 They are supervised, on behalf of the National Authorities, by officials of the Testing Authorities, that is 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
CB2 8DR

Tel No: 02080 265993
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
EH12 9FJ

Tel No 0131-244 8853
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/151/2 Rev	Protocol for Distinctness, Uniformity and Stability, Tests. Broccoli/Calabrese (<i>Brassica oleracea</i> L.). Published: 15.03.2017.
UPOV TG/151/4 Rev	Guidelines for the conduct of tests for Distinctness, Uniformity and Stability, Broccoli/Calabrese (<i>Brassica oleracea</i> L. convar. <i>botrytis</i> (L.) Alef. var. <i>cymosa</i> Duch.[including <i>Brassica oleracea</i> L. convar. <i>botrytis</i> (L.) Alef. var. <i>italica</i>]). Published 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 19.04.2002.
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 2008/91/EC, as amended, setting out implementing measures for the purposes of Article 7 of Council Directive 2002/55/EC (13 th 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. [Broccoli/Calabrese]
Council Regulation (EC) No. 2100/94	Council Regulation (EC) No. 2100/94 of 27 th July 1994 on Community Plant Variety Rights.

SECTION B - APPLICATION REQUIREMENTS**1 PURPOSE**

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

2 SCOPE

- 2.1 These procedures apply to all applications.

3 RESPONSIBILITIES

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

4 RECEIPT OF APPLICATIONS

- 4.1 The latest date for receipt of applications for acceptance of a variety onto the National List or for Plant Breeders' Rights is 15th February, 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 website at

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/714974/pbr-fees.pdf.

- 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 15th March 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 the 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

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 Broccoli/Calabrese.

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 *Brassica* 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, transplanting, fertiliser and spray application, use of irrigation, and control of pests and diseases.

5.3 CPVO characteristics that could be used for grouping:

Plant: number of stems (characteristic 1)
 Head: shape in longitudinal section (characteristic 17)
 Head: colour (characteristic 18)
 Time of harvest maturity (characteristic 30)
 Male sterility (characteristic 32)

- 5.4 Varieties known to be clearly different on any other discontinuous or continuous characteristic may be excluded from the trial and any further comparison with the candidate.
- 5.5 The tests are carried out using a grouped design, with a plot of each candidate and close control 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 transplants 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 candidates with distinctness problems in the first test cycle may be grown side by side with their close controls in the second or third test cycle.
- 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 test cycle, characters, as indicated in Section D5.1, are recorded on all candidates and their 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 test cycle, characters, as indicated in Section D5.1, are recorded on all candidates and their 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 is necessary, characters, as indicated in Section D5.1, are recorded on all candidates and their controls. The data for measured characters are analysed and, together with those from the first and second test 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 the applicant, 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, 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.

4 ORGANISATION

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

5 DUS CHARACTERISTICS TO BE ASSESSED

5.1 Routine Characteristics

The following table 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

Type of observation of characteristics:

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

A.1.1.1. plants

B.1.1.1.

C.1.1.1.

D.1.1.1. G denotes a grouping characteristic.

E.1.1.1. D denotes a characteristic used in the variety description.

F.1.1.1.

G.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.

BROCCOLI/CALABRESE CHARACTERISTICS ROUTINELY RECORDED IN DUS TESTS

Character Number			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
CPVO TP/151/2 2017	UPOV TG/151/4 2016	UK							
01DG QL VG	*01DG QL VG		Plant: number of stems	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=one 2=more than one	Clear visual difference or 1 state	Off-type standard and Uniformity score >5
02D QN MS/VG	*02D QN MS/VG		Plant: height (at harvest maturity)	DUS plot or single plants	At least 60 plants in total from 2 replicates or 40 plants in total from 2 replicates	Visual observation or visual score or measurement of single plants	1=very short 3=short 5=medium 7=tall 9=very tall	Clear visual difference or 2 states or COYD @ 5% for both 2 and 3 year tests	Off-type standard and Uniformity score >5 or COYU at 0.1% for both 2 and 3 year tests
03D QN VG	*03D QN VG		<u>Leaf: attitude (at beginning of head formation)</u>	DUS plot	At least 60 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
04D QN MS/VG	*04D QN MS/VG		<u>Leaf: length (including petiole)</u>	DUS plot or single plants	At least 60 plants in total from 2 replicates or 40 plants in total from 2 replicates	Visual observation or visual score or measurement of single plants	3=short 5=medium 7=long	Clear visual difference or 2 states or COYD @ 5% for both 2 and 3 year tests	Off-type standard and Uniformity score >5 or COYU at 0.1% for both 2 and 3 year tests
05D QN MS/VG	05D QN MS/VG		<u>Leaf: width</u>	DUS plot or single plants	At least 60 plants in total from 2 replicates or 40 plants in total from 2 replicates	Visual observation or visual score or measurement of single plants	3= narrow 5=medium 7=broad	Clear visual difference or 2 states or COYD @ 5% for both 2 and 3 year tests	Off-type standard and Uniformity score >5 or COYU at 0.1% for both 2 and 3 year tests
06D QN VG	*06D QN VG		Leaf: number of lobes	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=absent of very few 3=few 5=medium 7=many 9=very many	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
07D PQ VG	*07D PQ VG		<u>Leaf blade: colour</u>	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=green 2=grey green 3=blue green	Clear visual difference or 1 or 2 states	Off-type standard and Uniformity score >5

Section D

CPVO TP/151/2 2017	UPOV TG/151/4 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
08D QN VG	08D QN VG		Leaf blade: intensity of colour	DUS plot	At least 60 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
09D QL VG	09D QL VG		Leaf blade: anthocyanin coloration	DUS plot	At least 60 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
10D QN VG	10D QN VG		Leaf blade: undulation of margin	DUS plot	At least 60 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
11D QN VG	11D QN VG		Leaf blade: dentation of margin	DUS plot	At least 60 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
12DG QN VG	12DG QN VG		Leaf: blade blistering	DUS plot	At least 60 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 QL VG	13D QL VG		Petiole: anthocyanin coloration	DUS plot	At least 60 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
14D QN VG	14D QN VG		Petiole: length	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=very short 3=short 5=medium 7=long 9=very long	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
15D QN VG/MS	15D QN VG/MS		Head: length of branching at base (excluding stem)	DUS plot or single plants	At least 60 plants in total from 2 replicates or 40 plants in total from 2 replicates	Visual observation or visual score or measurement of single plants	1=very short 3=short 5=medium 7=long 9=very long	Clear visual difference or 2 states or COYD @ 5% for both 2 and 3 year tests	Off-type standard and Uniformity score >5 or COYU at 0.1% for both 2 and 3 year tests

Section D

CPVO TP/151/2 2017	UPOV TG/151/4 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
16DG QN VG	16DG QN VG		Head: size	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=very small 3=small 5=medium 7=large 9=very large	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
17DG QN VG	*17DG QN VG		Head: shape in longitudinal section	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=circular 2=transverse broad elliptic 3=transverse medium elliptic 4=transverse narrow elliptic	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
18DG PQ VG	*18DG PQ VG		Head: colour	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=cream 2=green 3=grey green 4=blue green 5=violet	Clear visual difference or 1 or 2 states	Off-type standard and Uniformity score >5
19D PQ VG	19D PQ VG		Head: intensity of colour	DUS plot	At least 60 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
20D QL VG	20D QL VG		Head: anthocyanin coloration	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=absent 9=present	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
21D QN VG	21D QN VG		Head: intensity of anthocyanin coloration	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=very weak 3=weak 5=medium 7=strong 9=very strong	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
22D QN VG	22D QN VG		Head: knobbling	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	3=fine 5=medium 7=coarse	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
23D QN VG	23D QN VG		Head: texture	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=very fine 3=fine 5=medium 7=coarse 9=very coarse	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
24D QN VG	24D QN VG		Head: firmness	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	3=loose 5=medium 7=firm	Clear visual difference or 2 states	Off-type standard and Uniformity score >5

Section D

CPVO TP/151/2 2017	UPOV TG/151/4 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
25D QL VG	25D QL VG		Head: bracts	DUS plot	At least 60 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
26D QL VG	26D QL VG		Plant: secondary heads (at harvest maturity)	DUS plot	At least 60 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
27D QN VG	27D QN VG		Plant: prominence of secondary heads (at harvest maturity)	DUS plot	At least 60 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
28D QL VG	28D QL VG		Flower: colour	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=white 2=yellow	Clear visual difference or 1 state	Off-type standard and Uniformity score >5
29D QN VG	28D QN VG		Flower: intensity of yellow colour	DUS plot	At least 60 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
30DG QN MG	*29DG QN MG		Time of harvest maturity (50% of plants)	DUS plot	At least 60 plants in total from 2 replicates	Visual observation or visual score	1=very early 3=early 5=medium 7=late 9=very late	Clear visual difference or 2 states	Off-type standard and Uniformity score >5
31D QN MG	31D QN MG		Time of beginning of flowering (50% plants with at least 10% of flowers)	DUS plot	At least 60 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
32DG QL VG	*32DG QL VG		Male sterility	DUS plot	At least 60 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

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			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
CPVO	UPOV	UK							

5.3 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**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 test 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 cycles, 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 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 cycles. 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 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 Broccoli/Calabrese entered for National List trials and Plant Breeders Rights.

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 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 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 to 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 Broccoli/Calabrese.

2 SCOPE

- 2.1 These procedures apply to all varieties of Broccoli/Calabrese 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. 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 at least 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 the assessment of uniformity of open-pollinated varieties, relative uniformity standards should be used.
- 6.5 For the assessment of uniformity of hybrid varieties, the total number of off-type plants recorded in the test, should not exceed the number 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, test cycles if, for all measured characters necessary for distinctness, the Combined Over years Uniformity (COYU) analysis is not significantly greater than that of the reference varieties at the 0.1% ($P=0.001$) significance level (see document 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 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.
- H.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.
- I.1.1.1. 1.3 All varieties on the UK National List and varieties on the EC Common Catalogue.
- J.1.1.1. 1.4 Varieties nominated by the authorities concerned where tests are done for other Member States.
- K.1.1.1. 1.5 Any varieties nominated by the applicant as being comparable i.e. known to be similar.
- L.1.1.1. 1.6 Any other varieties considered to be comparable i.e. known to be similar by the appropriate Test Centre or DUS Centre Group.

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.
- M.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).
- N.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.
- O.1.1.1. 2.4 Other available comparable varieties in common knowledge.