



Animal &  
Plant Health  
Agency

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

Broccoli/Calabrese

*Brassica oleracea* L. Broccoli/Calabrese

December 2022

## Contents

|                                                                 |    |
|-----------------------------------------------------------------|----|
| Section A – General Information .....                           | 2  |
| 1 Purpose                                                       | 1  |
| 2 Scope                                                         | 1  |
| 3 Responsibilities                                              | 1  |
| 4 Non-Compliance with the Protocol                              | 2  |
| 5 Responsibility for GM Releases                                | 2  |
| 6 Procedures for GM Varieties                                   | 2  |
| 7 Associated Documents                                          | 3  |
| Section B – Application Requirements .....                      | 4  |
| 1 Purpose                                                       | 4  |
| 2 Scope                                                         | 4  |
| 3 Responsibilities                                              | 4  |
| 4 Receipt of Applications                                       | 4  |
| 5 Receipt of Seed                                               | 4  |
| 6 Seed Quality Requirements                                     | 5  |
| 7 Seed Quantity                                                 | 5  |
| 8 Labelling Requirements, Including Provisions for GM Varieties | 6  |
| Section C – Growing Test Procedures .....                       | 7  |
| 1 Purpose                                                       | 7  |
| 2 Scope                                                         | 7  |
| 3 Responsibilities                                              | 7  |
| 4 Reference Varieties                                           | 7  |
| 5 Design of Tests                                               | 7  |
| 6 Records and Recording                                         | 8  |
| 7 Communications with the Applicant                             | 10 |

|                                                                                                                  |    |
|------------------------------------------------------------------------------------------------------------------|----|
| Section D – Summary of DUS Characteristics to be Assessed, Method of Assessment and Standards Applied .....      | 11 |
| 1 Purpose                                                                                                        | 11 |
| 2 Scope                                                                                                          | 11 |
| 3 Responsibilities                                                                                               | 11 |
| 4 Organisation                                                                                                   | 11 |
| 5 DUS Characteristics to be Assessed                                                                             | 11 |
| 5.2 Curly Kale Characteristics Routinely Recorded in DUS Tests                                                   | 13 |
| 5.3 Previously Approved Characteristics Not Routinely Recorded in DUS Tests                                      | 19 |
| Section E – Reference Seed Stock Maintenance and VCU Seed Stock Authentication Procedures, where applicable..... | 22 |
| 1 Purpose                                                                                                        | 22 |
| 2 Scope                                                                                                          | 22 |
| 3 Responsibilities                                                                                               | 22 |
| 4 Procedures for Reference Seed Stock Maintenance                                                                | 22 |
| 5 Procedures for VCU Seed Stock Authentication                                                                   | 23 |
| 6 Procedures for the Inclusion of New Common Knowledge Varieties into the Reference Collection                   | 23 |
| 7 Release of Reference Samples for Authorised Purposes                                                           | 23 |
| Section F – Procedures for Assessment of New Additional DUS Characters .....                                     | 25 |
| 1 Purpose                                                                                                        | 25 |
| 2 Scope                                                                                                          | 25 |
| 3 Responsibilities                                                                                               | 25 |
| 4 Reference Varieties                                                                                            | 25 |
| 5 Procedures                                                                                                     | 25 |
| Section G – Procedures for DUS Decisions.....                                                                    | 26 |
| 1 Purpose                                                                                                        | 26 |

|                                                  |    |
|--------------------------------------------------|----|
| 2 Scope                                          | 26 |
| 3 Responsibilities                               | 26 |
| 4 Reference Varieties                            | 26 |
| 5 Distinctness                                   | 26 |
| 6 Uniformity                                     | 27 |
| 7 Stability                                      | 28 |
| 8 DUS Report and Variety Description             | 28 |
| Appendix 1 – Reference Collection Varieties..... | 30 |
| 1 National Listing and Plant Breeders Rights     | 30 |

# Section A – General Information

## 1 Purpose

1.1 This Protocol sets out the procedures for conducting tests and assessments in relation to official examinations of DUS, maintenance of reference stocks of varieties of Broccoli/Calabrese entered for Variety Listing (VL) Trials and/or Plant Breeders' Rights (PBR).

## 2 Scope

2.1 These procedures apply to all varieties of *Brassica oleracea* L. var. *italica* Plenck, *Brassica oleracea* subvar. *cymosa* Duchesne, *Brassica oleracea* var. *cymosa* (Duchesne) 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](mailto: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:

|                           |        |               |
|---------------------------|--------|---------------|
| Vegetable DUS Test Centre |        |               |
| SASA                      |        |               |
| Roddinglaw Road           |        |               |
| Edinburgh                 | Tel No | 0131-244 8890 |
| 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. Where this protocol uses the word “should” for any action this is the method to be followed unless there are clear technical reasons 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/1/3                 | General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonised Descriptions of New Varieties of Plants (19.04.2002).                                                                                                          |
| UPOV TGP/8/4                | Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability (01.11.2019).                                                                                                                                                                        |
| UPOV TGP/9/2                | Examining Distinctness (29.10.2015).                                                                                                                                                                                                                                               |
| UPOV TGP/10/2               | Examining Uniformity (01.11.2019).                                                                                                                                                                                                                                                 |
| UPOV TG/151/5               | Guidelines for the conducts of tests for Distinctness, Uniformity and Stability Tests. Broccoli ( <i>Brassica oleracea</i> L. var. <i>italica</i> Plenck).<br>Published 30.10.2018                                                                                                 |
| UPOV TWC/26/14              | An Adjustment to the COYD Method When Varieties are Grouped Within the DUS Trial. 01.08.2008.                                                                                                                                                                                      |
| GB and NI Variety Lists     | The Seeds (National Lists of Varieties) Regulations 2001 (as amended); and The Seeds (Variety Lists) Regulations (Northern Ireland) 2020                                                                                                                                           |
| Plant Varieties Act 1997    | Plant Breeders' Rights Regulations 1998 and Plant Varieties Act 1997                                                                                                                                                                                                               |
| Plant Breeders' Rights 2019 | The Plant Breeders' Rights (Amendment etc.) (EU Exit) Regulations 2019 as amended by The Animal Health, Invasive Alien Species, Plant Breeders' Rights and Seeds (Amendment etc.) (EU Exit) Regulations 2019 and The Plant Breeders' Rights (Amendment) (EU Exit) Regulations 2020 |

# Section B – Application Requirements

## 1 Purpose

1.1 The purpose of this section is to identify the specific requirements for Variety Listing and/or Plant Breeders' Rights applications, as appropriate.

## 2 Scope

2.1 These procedures apply to all applications.

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

## 3 Responsibilities

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

## 4 Receipt of Applications

4.1 The latest date for receipt of applications for Variety Listing and/or for Plant Breeders' Rights is stated on the GOV website (<https://www.gov.uk/national-lists-of-agricultural-and-vegetable-crops>).

4.2 The procedures for the submission of Variety Listing and Plant Breeders' Rights applications, Technical Questionnaires (TQs) and for payment of administration fees can be obtained from APHA PVS at the address shown in Section A or on the GOV.UK website (<https://www.gov.uk/national-lists-of-agricultural-and-vegetable-crops>).

4.3 Applicants 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 is stated in the Seed Gazette. In the absence of exceptional circumstances, seed submissions received after this date will be refused. Instructions for the delivery of seed will be made available to applicants by APHA <https://www.gov.uk/national-lists-of-agricultural-and-vegetable-crops>

5.1 The latest date for receipt of seed is stated in the Seed Gazette. In the absence of exceptional circumstances, seed submissions received after this date will be refused. Instructions for the delivery of seed will be made available to applicants by APHA <https://www.gov.uk/national-lists-of-agricultural-and-vegetable-crops>



## 6 Seed Quality Requirements

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

6.2 The seed must not be chemically treated. Seed treatment, if required, 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 1<sup>st</sup> Test Cycle

3,000 or 9,000 seeds

### 7.2 2<sup>nd</sup> Test cycle

6,000 seeds if 3,000 seeds were provided in year 1

No seed if 9,000 seeds were provided in year 1

### 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, inside and outside the bag, with the following information:

- Applicant
- AFP number (if known)
- Breeder's Reference number or name
- Quantity of seed

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 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 it is used for any other purpose than the conduct of these procedures or the release of reference samples for authorised purposes. (See Section E7).

## 4 Reference Varieties

4.1 The principles governing the selection of reference varieties are set out in Appendix 1.

4.2 Seed of reference varieties will be supplied by the Test Centre.

## 5 Design of Tests

5.1 The 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 the risk is negligible.

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

5.3 The minimum duration of tests should normally be two independent growing cycles. The National List and Seeds Committee (NLSC) must be informed on any proposed changes to the number of cycles.

5.4 From information given in the Technical Questionnaire the candidate variety may be grown in plots and compared with varieties which are in the same classification for the following characters:

## UPOV characteristics that could be used for grouping:

Only Calabrese type varieties: Head: level of main head in relation to plant height (characteristic 13)  
Head: colour (characteristic 17)  
Time of harvest maturity (characteristic 23)  
Male sterility (characteristic 24)

5.5 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 must be sought. 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 and close control variety present in each replicate as follows:

|                                     |                |
|-------------------------------------|----------------|
| Number of replications              | 2              |
| Number of rows per plot             | 2              |
| Spacing between plot rows           | 0.8m (approx.) |
| 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.

5.7 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.

5.8 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 approximately 12 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 plant or plants resulting from an adverse reaction to husbandry practice are recorded but excluded from analysis.

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

6.5 If a third test cycle is necessary, characters, as indicated in Section D, 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 must be made of these at any time and a photographic record made.

## 7 Communications 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, method of observation and standards required for DUS assessment.

## 3 Responsibilities

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

## 4 Organisation

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

## 5 DUS Characteristics to be Assessed

### 5.1 Routine Characteristics

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

Note:

- \* a characteristic which must be examined according to the UPOV Guidelines.
- G a grouping 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 plants

Number of plants or sample size for assessment: Sample size of 60



## 5.2 Broccoli/Calabrese Characteristics Routinely Recorded in DUS Tests

| UPOV<br>TG/151/5 | Character                                              | Material<br>examined            | Number of<br>plants or<br>sample size for<br>assessment                                                    | Method of<br>assessment<br>and recording                                             | States of expression                                         | D Method and<br>Minimum<br>Distance<br>required                                                   | U Method:<br>standard<br>applied                                                                                    |
|------------------|--------------------------------------------------------|---------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------|---------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| 1                | <b>Plant: height (at harvest maturity)</b>             | DUS plot<br>or<br>single plants | At least 60<br>plants in total<br>from 2<br>replicates<br>or<br>40 plants in<br>total from 2<br>replicates | Visual<br>observation<br>or<br>visual score<br>or<br>measurement<br>of single plants | 1=very short<br>3=short<br>5=medium<br>7=tall<br>9=very tall | Clear visual<br>difference<br>or<br>2 states<br>or<br>COYD @ 5%<br>for both 2 and<br>3 year tests | Off-type<br>standard<br>and<br>Uniformity<br>score >5<br>or<br>COYU at<br>0.1%<br>for both 2<br>and 3 year<br>tests |
| 2                | <b>Leaf: attitude (at beginning of head formation)</b> | DUS plot                        | At least 60<br>plants in total<br>from 2<br>replicates                                                     | Visual<br>observation<br>or<br>visual score                                          | 1=erect<br>3=semi-erect<br>5=horizontal                      | Clear visual<br>difference<br>or<br>2 states                                                      | Off-type<br>standard<br>and<br>Uniformity<br>score >5                                                               |
| 3                | <b>Leaf: length (including petiole)</b>                | DUS plot<br>or<br>single plants | At least 60<br>plants in total<br>from 2<br>replicates<br>or<br>40 plants in<br>total from 2<br>replicates | Visual<br>observation<br>or<br>visual score<br>or<br>measurement<br>of single plants | 3=short<br>5=medium<br>7=long                                | Clear visual<br>difference<br>or<br>2 states<br>or<br>COYD @ 5%<br>for both 2 and<br>3 year tests | Off-type<br>standard<br>and<br>Uniformity<br>score >5<br>or<br>COYU at<br>0.1%<br>for both 2<br>and 3 year<br>tests |

| UPOV<br>TG/151/5 | Character                              | Material<br>examined            | Number of<br>plants or<br>sample size for<br>assessment                                                    | Method of<br>assessment<br>and recording                                             | States of expression                                               | D Method and<br>Minimum<br>Distance<br>required                                                   | U Method:<br>standard<br>applied                                                                                    |
|------------------|----------------------------------------|---------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| 4                | <b>Leaf: width</b>                     | DUS plot<br>or<br>single plants | At least 60<br>plants in total<br>from 2<br>replicates<br>or<br>40 plants in<br>total from 2<br>replicates | Visual<br>observation<br>or<br>visual score<br>or<br>measurement<br>of single plants | 1=very narrow<br>3= narrow<br>5=medium<br>7=broad                  | Clear visual<br>difference<br>or<br>2 states<br>or<br>COYD @ 5%<br>for both 2 and<br>3 year tests | Off-type<br>standard<br>and<br>Uniformity<br>score >5<br>or<br>COYU at<br>0.1%<br>for both 2<br>and 3 year<br>tests |
| 5                | <b>Leaf: number of lobes</b>           | DUS plot                        | At least 60<br>plants in total<br>from 2<br>replicates                                                     | Visual<br>observation<br>or<br>visual score                                          | 1=absent of very few<br>3=few<br>5=medium<br>7=many<br>9=very many | Clear visual<br>difference<br>or<br>2 states                                                      | Off-type<br>standard<br>and<br>Uniformity<br>score >5                                                               |
| 6                | <b>Leaf blade: colour</b>              | DUS plot                        | At least 60<br>plants in total<br>from 2<br>replicates                                                     | Visual<br>observation<br>or<br>visual score                                          | 1=green<br>2=grey green<br>3=blue green                            | Clear visual<br>difference<br>or<br>1 or 2 states                                                 | Off-type<br>standard<br>and<br>Uniformity<br>score >5                                                               |
| 7                | <b>Leaf blade: intensity of colour</b> | DUS plot                        | At least 60<br>plants in total<br>from 2<br>replicates                                                     | Visual<br>observation<br>or<br>visual score                                          | 3=light<br>5=medium<br>7=dark                                      | Clear visual<br>difference<br>or<br>2 states                                                      | Off-type<br>standard<br>and<br>Uniformity<br>score >5                                                               |

| UPOV<br>TG/151/5 | Character                               | Material<br>examined | Number of<br>plants or<br>sample size for<br>assessment | Method of<br>assessment<br>and recording    | States of expression                                                        | D Method and<br>Minimum<br>Distance<br>required | U Method:<br>standard<br>applied                      |
|------------------|-----------------------------------------|----------------------|---------------------------------------------------------|---------------------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------|-------------------------------------------------------|
| 8                | <b>Leaf blade: undulation of margin</b> | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 1=absent or very<br>weak<br>3=weak<br>5=medium<br>7=strong<br>9=very strong | Clear visual<br>difference<br>or<br>2 states    | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |
| 9                | <b>Leaf blade: dentation of margin</b>  | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 3=weak<br>5=medium<br>7=strong                                              | Clear visual<br>difference<br>or<br>2 states    | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |
| 10               | <b>Leaf: blade blistering</b>           | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 1=absent or very<br>weak<br>3=weak<br>5=medium<br>7=strong<br>9=very strong | Clear visual<br>difference<br>or<br>2 states    | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |
| 11               | <b>Petiole: anthocyanin coloration</b>  | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 1=absent or very<br>weak<br>3=medium<br>5=very strong                       | Clear visual<br>difference<br>or<br>1 state     | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |
| 12               | <b>Petiole: length</b>                  | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 1=very short<br>3=short<br>5=medium<br>7=long<br>9=very long                | Clear visual<br>difference<br>or<br>2 states    | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |

| UPOV<br>TG/151/5 | Character                                                                                  | Material<br>examined            | Number of<br>plants or<br>sample size for<br>assessment                                                    | Method of<br>assessment<br>and recording                                             | States of expression                                           | D Method and<br>Minimum<br>Distance<br>required                                                   | U Method:<br>standard<br>applied                                                                                    |
|------------------|--------------------------------------------------------------------------------------------|---------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|----------------------------------------------------------------|---------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| 13               | <b>Only Calabrese type varieties: Head: level of main head in relation to plant height</b> | DUS plot<br>or<br>single plants | At least 60<br>plants in total<br>from 2<br>replicates<br>or<br>40 plants in<br>total from 2<br>replicates | Visual<br>observation<br>or<br>visual score                                          | 1=low<br>2=medium<br>3=high                                    | Clear visual<br>difference<br>or<br>2 states<br>or<br>COYD @ 5%<br>for both 2 and<br>3 year tests | Off-type<br>standard<br>and<br>Uniformity<br>score >5<br>or<br>COYU at<br>0.1%<br>for both 2<br>and 3 year<br>tests |
| 14               | <b>Only Calabrese type varieties: Head: length of branching at base of main head</b>       | DUS plot<br>or<br>single plants | At least 60<br>plants in total<br>from 2<br>replicates<br>or<br>40 plants in<br>total from 2<br>replicates | Visual<br>observation<br>or<br>visual score<br>or<br>measurement<br>of single plants | 1=very short<br>3=short<br>5=medium<br>7=long<br>9=very long   | Clear visual<br>difference<br>or<br>2 states<br>or<br>COYD @ 5%<br>for both 2 and<br>3 year tests | Off-type<br>standard<br>and<br>Uniformity<br>score >5<br>or<br>COYU at<br>0.1%<br>for both 2<br>and 3 year<br>tests |
| 15               | <b>Head: diameter</b>                                                                      | DUS plot                        | At least 60<br>plants in total<br>from 2<br>replicates<br>or<br>40 plants in<br>total from 2<br>replicates | Visual<br>observation<br>or<br>visual score<br>or<br>measurement<br>of single plants | 1=very small<br>3=small<br>5=medium<br>7=large<br>9=very large | Clear visual<br>difference<br>or<br>2 states                                                      | Off-type<br>standard<br>and<br>Uniformity<br>score >5                                                               |

| UPOV<br>TG/151/5 | Character                                                                                                                          | Material<br>examined | Number of<br>plants or<br>sample size for<br>assessment | Method of<br>assessment<br>and recording | States of expression                                                                                      | D Method and<br>Minimum<br>Distance<br>required | U Method:<br>standard<br>applied          |
|------------------|------------------------------------------------------------------------------------------------------------------------------------|----------------------|---------------------------------------------------------|------------------------------------------|-----------------------------------------------------------------------------------------------------------|-------------------------------------------------|-------------------------------------------|
| 16               | <b>Only Calabrese type varieties: Head: shape in longitudinal section</b>                                                          | DUS plot             | At least 60 plants in total from 2 replicates           | Visual observation or visual score       | 1=circular<br>2=transverse broad elliptic<br>3=transverse medium elliptic<br>4=transverse narrow elliptic | Clear visual difference or 2 states             | Off-type standard and Uniformity score >5 |
| 17               | <b>Head: colour</b>                                                                                                                | DUS plot             | At least 60 plants in total from 2 replicates           | Visual observation or visual score       | 1=whitish<br>2=green<br>3=grey green<br>4=blue green<br>5=violet                                          | Clear visual difference or 1 or 2 states        | Off-type standard and Uniformity score >5 |
| 18               | <b>Head: intensity of colour</b>                                                                                                   | DUS plot             | At least 60 plants in total from 2 replicates           | Visual observation or visual score       | 3=light<br>5=medium<br>7=dark                                                                             | Clear visual difference or 2 states             | Off-type standard and Uniformity score >5 |
| 19               | <b>Only varieties with Head: color: <u>whitish, green, grey green or blue green</u>: Head: intensity of anthocyanin coloration</b> | DUS plot             | At least 60 plants in total from 2 replicates           | Visual observation or visual score       | 1=absent or very weak<br>2=weak<br>3=medium<br>4=strong                                                   | Clear visual difference or 2 states             | Off-type standard and Uniformity score >5 |
| 20               | <b>Only Calabrese type varieties: Head: knobbling</b>                                                                              | DUS plot             | At least 60 plants in total from 2 replicates           | Visual observation or visual score       | 3=fine<br>5=medium<br>7=strong                                                                            | Clear visual difference or 2 states             | Off-type standard and Uniformity score >5 |

| UPOV<br>TG/151/5 | Character                                                                      | Material<br>examined | Number of<br>plants or<br>sample size for<br>assessment | Method of<br>assessment<br>and recording    | States of expression                                                       | D Method and<br>Minimum<br>Distance<br>required | U Method:<br>standard<br>applied                      |
|------------------|--------------------------------------------------------------------------------|----------------------|---------------------------------------------------------|---------------------------------------------|----------------------------------------------------------------------------|-------------------------------------------------|-------------------------------------------------------|
| 21               | Head: diameter of flower bud                                                   | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 1=very small<br>3=small<br>5=medium<br>7=large<br>9=very large             | Clear visual<br>difference<br>or<br>2 states    | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |
| 22               | <u>Only Calabrese type varieties:</u> Plant:<br>development of secondary heads | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 1=absent or very<br>weak<br>3=weak<br>5=medium<br>7=strong                 | Clear visual<br>difference<br>or<br>2 states    | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |
| 23               | Time of harvest maturity (50% of<br>plants)                                    | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 1=very early<br>3=early<br>5=medium<br>7=late<br>9=very late               | Clear visual<br>difference<br>or<br>2 states    | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |
| 24               | Male sterility                                                                 | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 1=absent<br>9=present                                                      | Clear visual<br>difference<br>or<br>1 state     | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |
| 25               | Flower: colour                                                                 | DUS plot             | At least 60<br>plants in total<br>from 2<br>replicates  | Visual<br>observation<br>or<br>visual score | 1=white<br>2=whitish<br>3=light yellow<br>4=medium yellow<br>5=dark yellow | Clear visual<br>difference<br>or<br>1state      | Off-type<br>standard<br>and<br>Uniformity<br>score >5 |

## 5.3 Previously Approved Characteristics Not Routinely Recorded in DUS Tests

The following table summarises characteristics no longer used 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 | 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                |
|------|----|-------------------------------------------|-------------------|------------------------------------------------|------------------------------------|----------------------------------------------------------------|----------------------------------------|----------------------------------------------|
| N/A  | 1  | <b>Plant: number of stems</b>             | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 1=one<br>2=more than one                                       | Clear visual difference or<br>1 state  | Off-type standard and<br>Uniformity score >5 |
| N/A  | 9  | <b>Leaf blade: anthocyanin coloration</b> | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 1=absent<br>9=present                                          | Clear visual difference or<br>1 state  | Off-type standard and<br>Uniformity score >5 |
| N/A  | 16 | <b>Head: size</b>                         | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 1=very small<br>3=small<br>5=medium<br>7=large<br>9=very large | Clear visual difference or<br>2 states | Off-type standard and<br>Uniformity score >5 |
| N/A  | 23 | <b>Head: texture</b>                      | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 1=very fine<br>3=fine<br>5=medium<br>7=coarse<br>9=very coarse | Clear visual difference or<br>2 states | Off-type standard and<br>Uniformity score >5 |

| UPOV | 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             |
|------|----|-------------------------------------------------------------------|-------------------|------------------------------------------------|------------------------------------|--------------------------------|----------------------------------------|-------------------------------------------|
| N/A  | 24 | <b>Head: firmness</b>                                             | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 3=loose<br>5=medium<br>7=firm  | Clear visual difference or 2 states    | Off-type standard and Uniformity score >5 |
| N/A  | 25 | <b>Head: bracts</b>                                               | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 1=absent<br>9=present          | Clear visual difference or 1 state     | Off-type standard and Uniformity score >5 |
| N/A  | 26 | <b>Plant: secondary heads (at harvest maturity)</b>               | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 1=absent<br>9=present          | Clear visual difference or 1 state     | Off-type standard and Uniformity score >5 |
| N/A  | 27 | <b>Plant: prominence of secondary heads (at harvest maturity)</b> | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 3=weak<br>5=medium<br>7=strong | Clear visual difference or 2 states    | Off-type standard and Uniformity score >5 |
| N/A  | 28 | <b>Flower: colour</b>                                             | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 1=white<br>2=yellow            | Clear visual difference or 1state      | Off-type standard and Uniformity score >5 |
| N/A  | 29 | <b>Flower: intensity of yellow colour</b>                         | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 3=light<br>5=medium<br>7=dark  | Clear visual difference or 2 states    | Off-type standard and Uniformity score >5 |



| UPOV | 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             |
|------|----|---------------------------------------------------------------------------------|-------------------|------------------------------------------------|------------------------------------|-------------------------------|----------------------------------------|-------------------------------------------|
| N/A  | 31 | <b>Time of beginning of flowering (50% plants with at least 10% of flowers)</b> | DUS plot          | At least 60 plants in total from 2 replicates  | Visual observation or visual score | 3=early<br>5=medium<br>7=late | Clear visual difference or 2 states    | Off-type standard and Uniformity score >5 |

New Additional DUS Characteristics: Applicants can suggest new 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 and VCU Seed Stock Authentication Procedures, where applicable**

## **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 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 and the Variety Lists reviewed.

## 5 Procedures for VCU Seed Stock Authentication

Not applicable.

## 6 Procedures for the Inclusion of New Common Knowledge Varieties into the Reference Collection

6.1 When a new variety enters into common knowledge it must be included in the reference collection if seed is available. A request will be sent by the Test Centre to the maintainer of the variety and an official description will be requested from the Test 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.

6.2 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 be used to validate the sample of seed from the maintainer. The standards for the validation will be as for authentication of replacement seed (seed E4).

## 7 Release of Reference Samples for Authorised Purposes

7.1 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.

7.2 Provision of reference samples, other than in 7.1, to any other parties must be authorised by APHA.

# Section F – Procedures for Assessment of New Additional DUS Characters

## 1 Purpose

1.1 This section sets out the procedures for assessment of new additional DUS characters for varieties of Broccoli/Calabrese entered for Variety Listing trials and/or PBR.

## 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 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 standards used to assess distinctness, uniformity and stability of varieties of Broccoli/Calabrese.

## 2 Scope

2.1 These procedures apply to all varieties of *Brassica oleracea* L. var. *italica* Plenck, *Brassica oleracea* subvar. *cymosa* Duchesne, *Brassica oleracea* var. *cymosa* (Duchesne) DC entered for Variety Listing and/or Plant Breeders' Rights tests and those being tested on behalf of Foreign Authorities.

## 3 Responsibilities

3.1 The Test Centre is responsible for applying the criteria for DUS, set out in this procedure.

3.2 The Test Centre is responsible for producing the DUS report in accordance with these procedures and for ensuring that they are in accordance with UPOV guidelines.

## 4 Reference Varieties

4.1 Appendix 1 sets out which varieties are considered as reference varieties for these procedures.

## 5 Distinctness

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

5.2 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.

5.3 The distinctness standard applied for qualitative 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 at least two states (see table in section D 5.2) 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.

5.9 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.

5.10 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.

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 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 results 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:

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

1.3 All varieties with UK PBR.

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

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

1.6 Any varieties nominated by the applicant as being comparable i.e., known to be similar.

1.7 Any other varieties considered to be comparable i.e., known to be similar by the appropriate Test Centre or DUS Centre Group.

1.8 Other available comparable varieties in common knowledge.



© Crown copyright 2021

You may re-use this information (excluding logos) free of charge in any format or medium, under the terms of the Open Government Licence v.3. To view this licence visit [www.nationalarchives.gov.uk/doc/open-government-licence/version/3/](http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/) or email [PSI@nationalarchives.gsi.gov.uk](mailto:PSI@nationalarchives.gsi.gov.uk)

Data Protection:

For information on how we handle personal data visit [www.gov.uk](http://www.gov.uk) and search Animal and Plant Health Agency Personal Information Charter.

This publication is available at [www.gov.uk/government/publications](http://www.gov.uk/government/publications)

Any enquiries regarding this publication should be sent to us at

[www.gov.uk/apha](http://www.gov.uk/apha)

APHA is an Executive Agency of the Department for Environment, Food and Rural Affairs and also works on behalf of the Scottish Government, Welsh Government and Food Standards Agency to safeguard animal and plant health for the benefit of people, the environment and the economy.