Guidelines
for the Conduct of Test for
Distinctiveness, Uniformity and Stability

On

Broccoli

[*Brassica oleracea* (L.) var. *italica* Plenck]

(Valid from 1st May, 2024)

Protection of Plant Varieties and Farmers' Rights
Authority (PPV & FRA)
(A Statutory Body created by an Act of Parliament)
Government of India, New Delhi

Contents

Draft DUS Guidelines

(Valid from 1st May, 2024)

Broccoli [*Brassica oleracea* (L.) var. *italica* Plenck]

I. Subject

These DUS test guidelines shall apply to all varieties, hybrids and parental lines of Broccoli [*Brassica oleracea* (L.) var. *italic* Plenck)]

II. Seed material required

1. The Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA) shall decide when, where and in what quantity and quality the seed material is required for testing a variety denomination applied for registration under the Protection of Plant Varieties and Farmers Rights (PPV&FR) Act, 2001. Applicants submitting such material from a country other than India shall make sure that all customs and quarantine requirements stipulated under relevant national legislations and regulations are complied with. The minimum quantity of seeds for different categories to be packed, sealed, well labelled and submitted to the Authority by the applicant is as follows: -

New category	:	30 g in case of typical varieties and hybrids and 30 g for each of the parental lines of the hybrid (equal weighing 10 packets in single lot)
Extant (Variety of Common Knowledge) and Farmers category	:	15 g in case of typical variety or hybrid and 15 g for each of the parental lines of hybrid (equal weighing 5 packets in single lot)
Extant (Notified) category	:	10 g in case of typical variety or hybrid and 5 g for each of the parental lines of hybrid (equal weighing2 packets in single lot)

2. The seeds should meet the minimum requirements for germination capacity (including hard seeds minimum 65%), moisture content (maximum 7%) and purity (98%) as prescribed for certified seeds in India. The applicant shall also submit along with seed material a certified data on germination test made not more than one-month period prior to date of submission and shall possess the highest genetic purity, uniformity and phytosanitary standards.
3. The seeds supplied should be visibly healthy, not lacking in vigor or affected by any important pest or disease. In case, the seed is produced outside of the country, a phytosanitary certificate along with sample must be submitted. The seeds must not have undergone any treatment unless the Competent Authority allows or requests such treatment. If it has been treated, full details of the treatment must be given.
4. In case of hybrids, all the involved parents of the hybrid including maintainer parent of male sterility if any, along with the protocol for hybrid seed production also have to be supplied as per category above including seeds of hybrid.

5. The designated DUS testing centre shall maintain the variety during the testing period and after registration, it may be transferred to the reference variety collection that shall be maintained permanently, even if no DUS testing is done in any year.

III. Conduct of tests

1. The tests shall normally be conducted in two independent but similar growing seasons for varieties of new category and for varieties of extant (Varieties with Common Knowledge) and farmers' category for one season at two test locations with reference to the ecosystem of the candidate variety.
2. The field test shall be carried out under conditions favoring normal growth and expression of all test characteristics. The size of the plot should be such that plants or parts of plant could be removed for measurement and observation without prejudicing to the other observation on the standing plants until the end of the growing period. Each test shall include a minimum of 180 plants which should be divided among 3 replications. Separate plots for observation and for measurement can only be used if they have been subjected to similar environmental conditions. All the replications shall be sharing similar environmental conditions of the test location.
3. **The test plot design shall be as follows:**

Experimental Design	:	Randomized Block Design
No. of replications	:	03
Sowing time	:	2nd fortnight of September
Date of Transplanting	:	3o days after sowing
Crop spacing	:	60 x 45 cm
Crop management practices	:	As per standard procedure
Plot size	:	18 sq. me (5.0 m x 3.6 m)
No. of plants per replication	:	60 plants (6 rows per replication and each row having 10 plants; each plot should not have less than 50 plants)

4. Observations should not be recorded on plants in border rows.
5. Additional test protocol for special purpose shall be established by the PPV&FR Authority.

IV. Methods and observations

1. The characteristics described in the Table of characteristics (see section VII) shall be used for testing of candidate varieties for DUS.
2. For the assessment of Distinctiveness and Stability observations shall be made on 30 plants or parts of 30 plants which should be divided among 3 replications (10 plants per replication).
3. For the assessment of Uniformity of characteristics on the plot as a whole (visual assessment by a single observation of a group of plants or parts of plants), a population standard of 1% with an acceptance probability of at least 95% should be applied. In the case of a sample size of 180 plants, the number of off-types should not exceed 3.
4. All observations (unless otherwise indicated) on the plant and leaf should be made on plant fully developed in the vegetative stage.

5. All the leaf observations shall be observed on the 5th leaf in second whorl from inner side.
6. Leaf width should be measured at the broadest part of the leaf.
7. The head characters except specified should be recorded at fully developed compact stage.
8. For the assessment of colour characteristics, the latest Royal Horticultural Society (RHS) colour chart shall be used.
9. Stage of recording observations on specific characteristics shall be as follows:

Stage Code	**Description**
10	Seedling stage
40	Active vegetative growth before headinitiation
50	Appearance of head
60	50% of plants in the heading stage
70	50% of plants in marketable maturity
90	50% of plants in flowering stage

V. Grouping characteristics

1. The candidate varieties for DUS testing should be divided into groups to facilitate the assessment of Distinctiveness. Characteristics known from experience not to vary, or to vary only slightly within a variety and which in their various states are fairly evenly distributed across all varieties in the collection are suitable for grouping purposes.
2. Grouping characteristics are: -
   a) Seedlings: Anthocyanin coloration of hypocotyls (Characteristic 1)
   b) Stem: Branches or spears on main stem at head maturity (Characteristic 2)
   c) Leaf: Attitude (Characteristic 3)
   d) Head: Colour (Characteristic 15)
   e) Head bud: Texture (Characteristic 18)
   f) Maturity:50% plants attain harvest maturity (Characteristic 24)
   g) Flower: Male sterility (Characteristic 27)

VI. Characteristics and symbols

1. To assess Distinctiveness, Uniformity and Stability the characteristics and their states as given in the Table of Characteristics should be used.
2. "Notes" (1 to 9 as shown in table VII) shall be used to describe the state of each character for the purposes of digital data processing and these notes shall be given opposite to the states of different characteristics.
3. Legend

(*) Characteristics that shall be observed during every growing season on all varieties shall always be included in the description of the variety, except when the state of expression of a preceding phenological characteristic or by environmental conditions of the testing region. Under such exceptional situations, adequate explanation shall be protected.

(+) See Explanations for the Table of Characteristics in Section VIII. It is to be noted that for certain characteristics the plant parts on which observations to be taken are given in the explanation of figure(s) for clarity and not for the colour variation.

(ð) This symbol indicates representation of RHScolour chart number.

4**.** Type of assessment of characteristics indicated in column 7 of Table of characteristics is as follows:

- MG: Measurement by a single observation of a group of plants or parts of plants
- MS: Measurement of a number of individual plant or parts of plants
- VG: Visual assessment by a single observation of a group of plants or parts of plants
- VS: Visual assessment by observations of individual plant or parts of plants

VII. Table of characteristics

Sl. No.	Characteristics	Status	Note	Example varieties	Stages of observation	Type of Assessment
1	Seedling: Anthocyanin coloration of hypocotyls	Absent	1	Pusa KTS-1 Palam Kanchan	10	VG
		Present	9	Pusa Purple Broccoli-I Palam Vichitra		
2	Stem: branches or spears on main stem at head maturity	Absent	1	Pusa Purple Broccoli-1	40	VG
		Present	9	Punjab Broccoli-1 Pusa KTS-1		
3	Leaf attitude (at the beginning of head formation)	Erect	1	Palam Vichitra		
		Semi-erect	5	Pusa KTS-1 Pusa Purple Broccoli-1		
		Horizontal	9	Punjab Broccoli-1		
4	Leaf length with petiole (cm)	Short (<35 cm)	3	Sher-E-Kashmir	40	MS
		Medium (35-45 cm)	5	Palam Samridhi Pusa Purple Broccoli-1		
		Long (>45 cm)	7	Palam Vichitra, Palam Kanchan,		
5	Leaf width (cm)	Narrow (<15 cm)	3	Sher-E-Kashmir Pusa Purple Broccoli-1	40	MS
		Medium (15- 25 cm)	5	Palam Haritika		
		Broad (>25 cm)	7	Palam Kanchan		
6	Leaf: No. of lobes	Absent	1	Pusa Purple Broccoli-1 Palam Kanchan	40	VG
		Medium (1-3)	5	Palam Samridhi		

		High (>3)	9	Palam Haritika		
7	Leaf: Blade Colour	Green	3	Sher-E-Kashmir(GG Dark B/133A) Pusa KTS-1 (GG/133 A)	40	VG
		Grey green	5	Palam Samridhi (GG 136A)		
		Dark green	7	Palam Haritika		
		Purplish Green	9	Pusa Purple Broccoli-1 (GG 136B) Palam Vichitra (137 B)		
8	Leaf Blade: Dentation of Margin	Weak	3	Sher-E-KashmirPusa KTS-1	40	VG
		Medium	5	Palam Kanchan		
		Strong	7	-		
9	Leaf Blade: Undulation of Margin	Weak	3	Pusa Purple Broccoli-1 Palam Vichitra	40	VG
		Medium	5	Pusa KTS-1 Palam Samridhi		
		Strong	7	Palam Haritika		
10	Leaf: Petiole length (cm) (without lamina)	Short (<5 cm)	3	Pusa Purple Broccoli-1	40	VG
		Medium (5-10 cm)	5	Palam Haritika		
		Long (>10 cm)	7	Sher-E-Kashmir		
11	Petiole Anthocyanin Coloration	Absent	1	Pusa KTS-1	40	VG
		Present	9	Pusa Purple Broccoli-2		
12	Plant height (At 50% plant harvest maturity)	Short (<45 cm)	3	DC Brocco-20-8	70	MS
		Medium (45-65 cm)	5	Pusa KTS-1		
		Tall (>65 cm)	9	Palam Vichitra Palam Kanchan		
13	Head shape in longitudinal section	Circular	3	Palam Vichitra	70	VG
		Transverse broad elliptic	5	Palam Kanchan		
		Transverse medium elliptic	7	Pusa Purple Broccoli-1		
		Transverse narrow elliptic	9	Sher-E-Kashmir, Punjab Broccoli		
14	Head: diameter	Small (<10 cm)	3	Sher-E-Kashmir, Punjab Broccoli	70	VG
		Medium (10-15 cm)	5	Pusa KTS-1		
		Large (>15 cm)	7	Palam Vichitra		
15	Head colour	Yellowish green	3	Palam Kanchan (YGG N 144)	70	VG
		Green	5	Pusa KTS-1 (GG/N 137 B)		

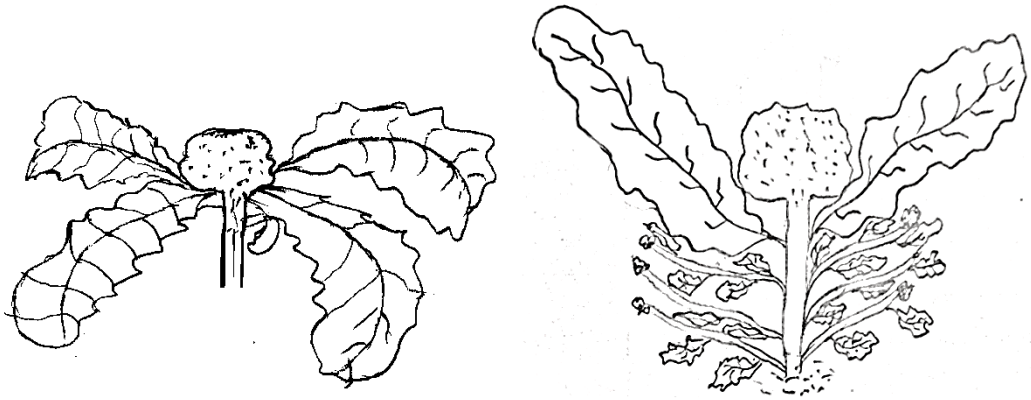
		Purple	7	Pusa Purple Broccoli- 1 (PPG/79A) Palam Vichitra (PG/N77A)		
16	Head: intensity of colour	Light	3	Palam Kanchan	70	VG
		Medium	5	Punjab Broccoli-1 Palam Vichitra		
		Dark	7	Pusa Purple Broccoli-1 Palam Haritika		
17	Head: knobbing (knobs/ head)	Fine	3	Palam Vichitra Palam Kanchan	70	VG
		Medium	5	Pusa KTS-1 Pusa Purple Broccoli-1		
		Coarse	7	Punjab Broccoli-1		
18	Head: texture	Fine	3	Palam Kanchan Palam Vichitra	70	VG
		Medium	5	Pusa Purple Broccoli-1		
		Coarse	7	Punjab Broccoli-1		
19	Head: firmness	Loose	3	Sher-E-Kashmir Punjab Broccoli-1	70	VG
		Medium	5	Pusa KTS-1 Pusa Purple Broccoli-1		
		Firm	7	Palam Vichitra Palam Kanchan		
20	Peduncle length from branching base (Excluding Stem)	Short (<5 cm)	3	Palam Vichitra Palam Kanchan	70	MS
		Medium (5-10 cm)	5	Palam Haritika Pusa Purple Broccoli-1		
		Long (11-15 cm)	7	Pusa KTS-1 Palam Samridhi		
21	Head size: (on the basis of net head weight)	Small (<200 g)	3	Sher-E-Kashmir Punjab Broccoli-1	70	VG
		Medium (200-400 g)	5	Pusa Purple Broccoli-1		
		Large (>400 g)	7	Palam Vichitra Palam Kanchan		
22	Plant: secondary heads (at harvest maturity)	Absent	1	Pusa Purple Broccoli-1 Palam Vichitra Palam Kanchan	70	VG
		Present	9	Sher-E-Kashmir Palam Samridhi		
23	Plant: Prominence of secondary heads	Absent	1	Pusa Purple Broccoli-1	70	VG
		Weak	3	Sher-E-Kashmir Pusa KTS-1		
		Medium	5	Palam Samridhi Punjab Broccoli-1		
		Strong	7	Palam Haritika		
24	Time of harvest: maturity 50%	Very early (<100 DAS)	1	Sher-E-Kashmir Punjab Broccoli-1	70	MG
		Early (100-115	3	Pusa KTS-1		

	of plants	DAS)		Palam Samridhi Pusa Purple Broccoli-1		
		Medium (116-130 DAS)	5	-		
		Late (131 -145 DAS)	7	Palam Vichitra Palam Haritika		
		Very late (>145 DAS)	9	Palam Kanchan		
25	Time of beginning of flowering (at least 10 % plant in flowering)	Early (<130 DAS)	3	Punjab Broccoli-1 Pusa Purple Broccoli-1	90	MG
		Medium (130-160 DAS)	5	Pusa KTS-1 Palam Samridhi		
		Late (>160 DAS)	7	Palam Vichitra Palam Kanchan		
26	Flower colour	White	1	DC-Brocco-20-5	90	VG
		Yellow	7	Palam Samridhi Punjab Broccoli-1		
27	Male sterility	Absent	1	Pusa KTS-1 Palam Samridhi Pusa Purple Broccoli-1	90	VG
		Present	9	DC-Brocco64A, DC-Brocco-15A		

Annexure-II

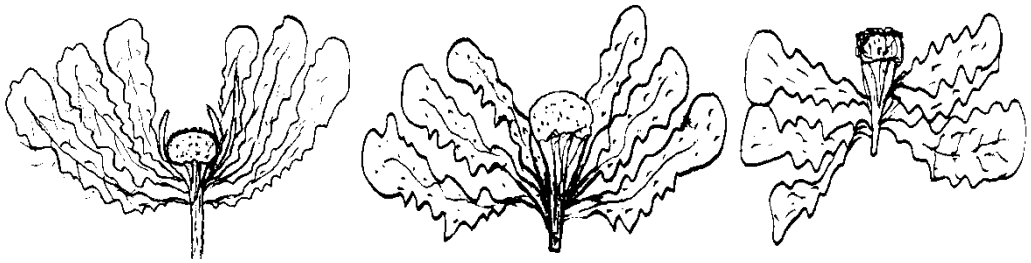
Diagrammatic explanation for individual characteristics

Ch. 2. Stem: branches or spears on main stem at head maturity



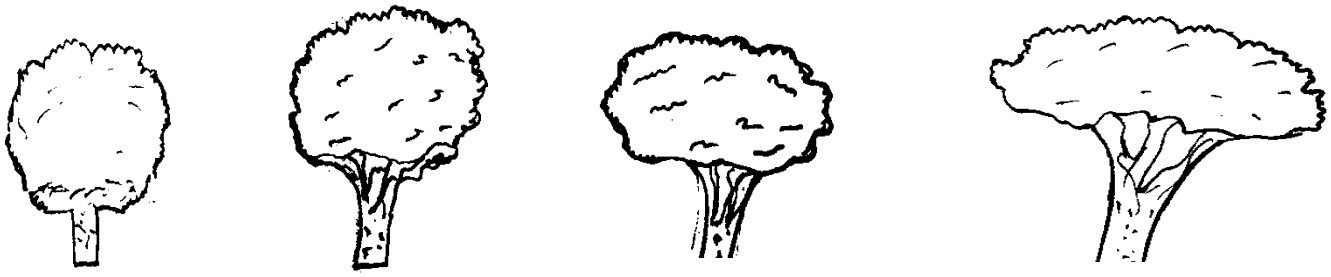
Absent (1) **Present (9)**

Ch. 3. Leaf attitude (at beginning of head formation)



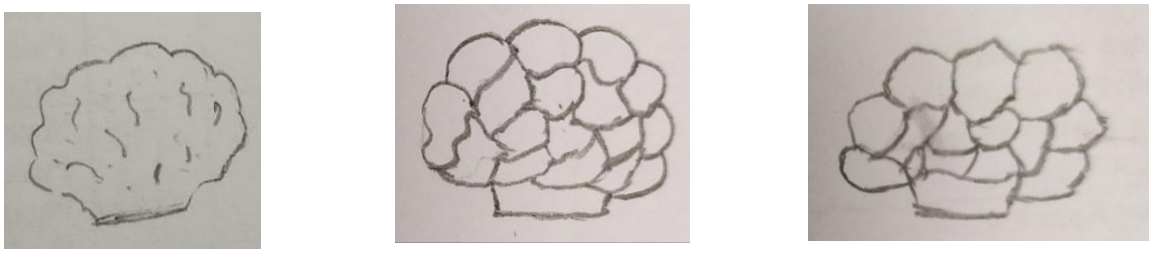
Erect (1) **Semi-erect (5)** **Horizontal (9)**

Ch 13: Head shape in longitudinal section



Circular (3) **Transverse broad elliptic (5)** **Transverse medium elliptic (7)** **Transverse narrow elliptic (9)**

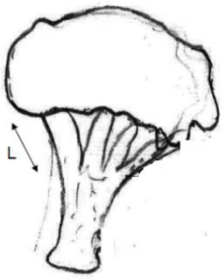
Ch. 17 Head: knobbing (knobs/head)



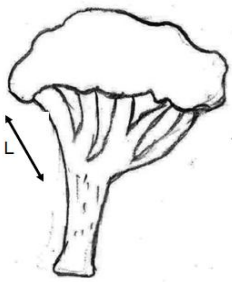
Fine (3) **Medium (5)** **Coarse (9)**

Ch 20: Pedunclelength atbranching at base (excluding stem)

Short (3)



Medium (5)



Long (7)

IX. Working Group details:

The test guidelines developed by the task force (5/2006) constituted by the PPV & FR Authority for Broccoli [*Brassica oleracea* (L.) var. *italica*Plenck] with consultation by Nodal officer, ICAR-IARI, New Delhi. The Technical inputs also provided by the PPV & FR Authority.

1.	**Dr A. S. Dhatt** Director of Research (Horticulture and Food Science) Punjab Agricultural University Ludhiana Punjab	Chairman
2.	**Dr. Hardayal Singh Kanwar** EX- Professor& Head Department of Vegetable Science Dr. Y. S. Parmar University of Horticulture & Forestry Nauni, Solan, HP	Member
3.	**Dr. T. P. Malik** Professor, Department of Vegetable Science CCSHAU, Hisar Haryana	Member
4.	**Dr. S. R. Sharma** EX- Head & Principal Scientist ICAR-IARI, Regional Station, Katrain	Member
5.	**Dr. B. S. Tomar** Head, Division of Vegetable Science ICAR-IARI, Pusa Campus, New Delhi	PI of DUS Project

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Annexure-III

Colour photographs of important traits of Broccoli for DUS characterization

Ch. 1. Seedling: Anthocyanin coloration of hypocotyls

Absent (1) **Present (9)**

Ch. 2. Stem: branches or spears on main stem at head maturity

Absent (1) **Present (9)**

Ch. 3. Leaf attitude at beginning of head formation

		
Erect (1)	**Semi-erect (5)**	**Horizontal (9)**

Ch. 4. Leaf length (cm) with petiole

		
Short (3)	**Medium (5)**	**Long (7)**

Ch. 6. Leaf: Number of lobes

		
Absent (1)	**Medium (5)**	**High (9)**

Ch. 11. Petiole Anthocyanin Coloration

Absent (1) **Present (9)**

Ch.14. Head: diameter

Ch. 15. Head colour

		
Yellowish green (3)	**Green(5)**	**Purple(7)**

Ch. 19. Head firmness

		
Loose(3)	**Medium(5)**	**Firm(7)**

Ch 20: Peduncle length at branching at base (excluding stem)

Short (3)

Medium(5)

Long (7)

Ch. 22. Plant: Secondary head (at harvest maturity)

Absent (1)

Present (9)

Ch. 23. Plant: Prominence of secondary head

Absent (1)

Weak (3)

Medium (5)

Strong (7)

Ch.26. Flower: Colour

White (1)

Yellow (7)

Ch.27. Flower: Male sterility

Absent (1) **Present (9)**

Absent (1) **Present (9)**