

Guidelines
for the Conduct of Test for
Distinctiveness, Uniformity and Stability
on varieties of
Kale (Brassica oleracea L. var. acephala)

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

Contents

- I.** Subject
- II.** Planting Material Required
- III.** Conduct of Tests
- IV.** Assessment of Distinctiveness, Uniformity and Stability
- V.** Method of Observation
- VI.** Grouping of Varieties
- VII.** Characteristics and Symbols
- VIII.** Table of Characteristics
- IX.** Explanation for the Table of Characteristics
- X.** Working Group Details
- XI.** DUS Testing Centres

I. Subject

These DUS test guidelines shall apply to all cultivated varieties of Kale (*Brassica oleracea* L. var. *acephala*).

II. Planting material required

1. The Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA) should decide when, where, of what quality and in what quantity the planting material is required for testing a variety denomination applied for registration under the Protection of Plant Variety and Farmers' Rights (PPV & FR) Act, 2001. Applicants submitting such plant material from a country other than India should act in accordance with all customs and quarantine requirements stipulated under relevant national legislations and regulations. As a minimum the applicant needs to submit 5 g seed of the variety to be tested.
2. The seed must adhere to the minimal standards for germination, health, and moisture content set by the appropriate authority. The germination capacity should be as high as possible and be specified by the applicant in circumstances where the seed is to be stored.
3. The seed should be healthy, vigorous, and unaffected by any significant pest or disease.
4. Unless authorized or requested by the competent authorities, the seeds should not have undergone any treatment that would influence the expression of the characteristics of the genotype. If it has been treated, all information regarding the procedure must be provided.

III. Conduct of tests

1. Normally, DUS tests should last for at least two separate growth cycles. The two different plantings should represent the two independent growing cycles.
2. The tests should be carried out under favorable conditions ensuring satisfactory growth and expression of the relevant characteristics of the variety and for its evaluation.
3. The dimensions of the plot must allow for the removal of plants or plant parts for measurement and counting without affecting the ongoing observations required until the end of evaluation period. Each test should consist of at least 90 plants, distributed among three replications.
4. The additional test protocol for examining relevant characteristics may be established by PPV & FRA.

Test plot design

1. Plot size: 4.5m x 1.5m

2. Spacing: 45 cm x 45 cm
3. No. of replications: Three
4. No. of plants per replication: 30

IV. Assessment of Distinctiveness, Uniformity and Stability

1. The characteristics described in the table of characteristics (see section VIII) shall be used for the DUS testing of varieties.
2. For the assessment of distinctiveness and stability, observation shall be made on 30 plants or parts of plants (10 plants in each replication).
3. For the assessment of uniformity of characteristics, a population standard deviation of 5% with the acceptance probability of at least 95% should be permitted.
4. In the case of a sample size of 90 plants, the maximum number of off-types allowed will be one.

V. Method of observation

- a) **MG:** Measurement of a group of plants or plant parts based on a single observation
- b) **MS:** Measurement of an individual plant or plant part based on a single observation
- c) **VG:** Visual assessment of a group of plants or plant parts based on a single observation
- d) **VS:** Visual assessment of an individual plant or plant part based on a single observation

For the assessment of colour characteristics, edition V of the Royal Horticultural Society (RHS) colour chart shall be considered

Methodology used for important traits

1. **Leaf traits:** All leaf traits were observed on fifth leaf from top of the plant.
2. **Leaf length:** It was measured from the tip of leaf till the point where main blade ends on the petiole, excluding the wing like structures.
3. **Leaf width:** It was measured across the widest part of the leaf.
4. **Leaf petiole length:** The length of petiole was measured by placing the foot ruler at the end of petiole up till the point where true leaf blade starts, excluding the wing like structures on the petiole.
5. **Leaf petiole width:** The portion of petiole from where it detaches from the stem was measured to estimate petiole width.

VI. Grouping of varieties

1. For the assessment of distinctiveness, the candidate varieties for DUS testing shall be separated into groups. The best characteristics for grouping are those that, according to experience, don't fluctuate much or only slightly within a variety and that, in their various stages, are distributed uniformly throughout all the varieties in the collection.
2. Grouping characteristics are those in which the documented states of expression, even if produced at different locations, can be used, either individually or in combination with other such characteristics to (a) select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctiveness; and (b) to organize the growing trial so that similar varieties are grouped together.
3. The following characteristics have been agreed upon for grouping of varieties. It is recommended that the competent authorities use the following characteristics for grouping of kale varieties.
 1. **Plant** : Stem color (Characteristic 17)
 2. **Plant** : Growth habit (Characteristic 21)
 3. **Plant** : Main stem branching (Characteristic 22)
 4. **Leaf** : Apex shape (Characteristic 5)
 5. **Leaf** : Puckering (Characteristic 12)
 6. **Leaf** : Presence of lobes (Characteristic 14)

VII. Characteristics and symbols

1. The characteristics and their states as listed in the table of characteristics (Section VIII) shall be utilized to evaluate kale varieties for distinctiveness, Uniformity, and Stability.
2. Many descriptors which are continuously variable shall be recorded on a 1-9 scale. Absence/ Presence of characters will be scored as:
 - a. 1 Absent
 - b. 9 Present
3. When a descriptor is not applicable, 1 is used as the descriptor value. For instance, if an accession lacks flower scent, the following descriptor would receive a score of "1".

4. Legend

- a. (*) Characteristics that should be observed during every growing season in all varieties and should always be included in the description of the variety. In exceptional cases, wherein the state of expression of any of these characters is rendered impossible either by the environmental vagaries or any other reason, adequate elucidation should be provided.
- b. (+) See diagrammatic explanations on the table of characteristics in Section IX. It is to be noted that for certain characteristics, the plant parts on which observations are to be recorded should be given in the explanation or figure(s) for clarity on the table of characteristics in Section VIII.
- c. The optimum stage to observe each characteristic during the growth and development of a plant is identified by a code number in the sixth column of the table of characteristics. The following is a description of the pertinent growth stages corresponding to these code numbers:

VIII: Table of characteristics

S. No.	Characteristics	States	Note	Example Variety	Stage of observation	Type of assessment
1	2	3	4	5	6	7
1. (+)	Seedling: Leaf pubescence	Absent	1	IC-594205	S1	VS
		Present	9	IC-0650706, IC-0650708		
2. (+)	Leaf: Colour	Light green (Green 138A)	1	IC-0650682	S2	VS
		Dark green (Green N138B)	2	IC-0650694		
		Yellow green (Yellow green147B)	3	IC-594205		
3. (*)(+)	Leaf: Midrib colour	Green (128D)	1	IC-0650674	S2	VS
		Purple (77B)	2	IC-0650679		
4. (*)(+)	Leaf: Shape	Elliptic	1	IC-594204	S2	VS
		Lanceolate	2	IC-0650698		
		Obovate	3	IC-0650697		
		Orbicular	4	IC-0650671		
		Lobed	5	IC-0650708		
5. (*)(+)	Leaf: Apex shape	Round	1	IC-0650697	S2	VS
		Obtuse	2	IC-0650691		
		Acute	3	IC-0650669		
6.		Short (<15)	3	IC-0650693, IC-0650697	S2	MS

(+) (+)	Leaf: Length (cm)	Medium (15-25)	5	IC-0650713, IC-0650677		
		Long (>25)	7	IC-0650695, IC-594205		
7. (+)	Leaf: Width (cm)	Narrow (<15)	3	IC-594208	S2	MS
		Medium (15-20)	5	IC-594201		
		Broad (>20)	7	IC-0650695		
8. (+)	Leaf: Incisions	Absent	1	CITH-KC-3, HW-5	S2	VS
		Present	9	IC-594203, Pusa Sag-2		
9.	Leaf: Depth of incisions	Absent	1	IC-0650671	S2	VS
		Shallow	2	IC-0650681		
		Medium	3	IC-594205		
		Deep	4	Pusa Sag-1		
10. (+)	Leaf: Waviness	Absent	1	IC-0650670	S2	VS
		Present	9	IC-0650708, Pusa Sag-4		
11. (+)	Leaf: Margin	Entire	1	IC-0650697	S2	VS
		Crenate	2	IC-594205		
		Lobed	3	Pusa Sag-1		
12. (*) (+)	Leaf: Puckering	Absent	1	IC-0650689	S2	VS
		Weak	3	IC-0650676		
		Strong	5	IC-0650697, IC-0650673		
13. (+)	Leaf: Recurvature of margin	Weak	3	IC-0650684	S2	VS
		Medium	5	IC-0650676		
		Strong	7	IC-594197		
14. (*) (+)	Leaf: Presence of lobes	Absent	1	IC-0650669	S2	VS
		Present	9	Siberian, IC-0650708, Pusa Sag-1,2,3,4		
15. (+)	Leaf: Petiole length (cm)	Short (<10)	3	IC-0650671, IC-0650713	S2	MS
		Medium (10-20)	5	Siberian		
		Long (>20)	7	IC-0650711		
16. (+)	Leaf: Petiole width (mm)	Narrow (<7)	3	IC-0650693, IC-0650671	S2	MS
		Medium (7-10)	5	IC-0650683, IC-0650682		
		Broad (>10)	7	IC-0650692		
17. (*) (+)	Plant: Stem colour	Green (Green 38B)	1	IC-0650688	S2	VS
		Purple (Purple 77A)	2	IC-594197		
18.	Leaf: Number of leaves per plant	Few (<40)	3	IC-0650679, IC-0650678	S2	MS
		Several (40-80)	5	IC-0650697, Pusa Sag-1		
		Many (>80)	7	IC-0650693, IC-0650671		

19. (+)	Plant: Height (cm)	Short (<40)	3	Pusa Sag – 2	S2	MS
		Medium (40-60)	5	IC-0650682		
		Tall (>60)	7	IC-0650697, IC-0650671		
20. (+)	Plant: Canopy diameter (cm)	Small (<30)	3	IC-0650671	S2	MS
		Medium (30-50)	5	IC-0650697, IC-0650693, IC-0650695		
		Large (>50)	7	IC-0650684, IC-0650709		
21. (+) (*)	Plant: Growth habit	Erect	1	IC-0650671, IC-0650697	S2	VS
		Semi-erect	2	IC-0650682		
		Prostrate	3	Pusa Sag-1		
22. (*) (+)	Plant: Main stem branching	Absent	1	IC-0650682	S2	VS
		Present	9	IC-0650671		
23. (+)	Inflorescence: Axis growth	Condensed	1	IC-0650678	S4	VS
		Elongate	9	IC-0650676		
24. (+)	Floral stalk branching	Sparse	3	Siberian, Pusa Sag-1	S4	VS
		Moderate	5	IC-0650671, IC-0650705		
		Profuse	7	IC-0650697, IC-0650699		
25. (*)	Floral bud: Colour	Green (Green N138B)	1	IC-0650692	S4	VS
		Yellow (Green yellow1C)	2	IC-0650709		
		Purple (purple N77B)	3	Pusa Sag-2		
26. (+)	Flower: Stalk colour	Green	1	IC-594205, IC-0650671	S4	VS
		Purple	2	Pusa Sag-2, IC-0650712		
27. (*) (+)	Siliqua: Attitude	Upright	3	IC-0650670, IC-0650682	S5	VS
		Horizontal	5	IC-0650684, IC-0650693		
		Drooping	7	IC-0650671		
28. (+)	Siliqua: Length (cm)	Short (<4)	3	IC-594207	S5	MS
		Intermediate (4-8)	5	IC-0650694, IC-0650669, IC-594204		
		Long (>8)	7	IC-0650686, IC-0650688		
29. (+)	Siliqua: Colour before drying	Green (Green 138A)	1	IC-0650669	S5	VS
		Yellow (Yellow green N144A)	2	IC-0650673		
		Violet (Violet-Blue 92A)	3	Pusa Sag-3		
		Purple	4	IC-0650708		

		(Red purple 65A)				
30.	Seeds: Number per siliqua	Few (<10)	3	Pusa Sag-1, IC-0650713	S6	MS
		Several (10-20)	5	IC-0650697, IC-0650671 IC-0650685		
		Many (>20)	7	IC-0650684		
31.	Seed: 1000 seed weight (g)	Light (<3)	3	Pusa Sag-4, IC-0650708	S6	MS
		Medium (3-6)	5	IC-0650691, IC-0650682, IC-0650671		
		Heavy (>6)	7	IC-0650697, IC-0650711		
32. (+)	Seed: Colour	Brown (Brown 200C)	1	IC-0650713, IC-0650671	S6	VS
		Grey (Grey-Orange 177A)	2	IC-0650697, IC-594207		
		Black (Black 202A)	3	Siberian, Pusa Sag-4, IC-0650708		

Table: Description and growth stages for the recording of traits

Stage	Growth Stages	Description	Time Period	Stage
1	Seedling	Observations on seedling leaf margin	Mid July – Mid August	S1
2	Vegetative	Observations on plant and fully expanded leaves before and at harvest	October	S2
3	Reproductive	Observations on inflorescence characteristics when the plants are in full bloom	March – May	S3
4	Fruit	Observations on siliqua characteristics	May – June	S4
5	Seed	Observations at 5 – 6 % moisture content according to ISTA rules	July – August	S5

S1: It is the stage of plant life spent in the nursery from the day of sowing till its transplanting to the main field. In Kashmir conditions, it occurs from mid-July to mid-August.

S2: It is the stage of plant life that is spent in vegetative state. It starts at the establishment of transplants in the main field till the cessation of new growth normally by the end of December. The most luxuriant vegetative growth is observed during October.




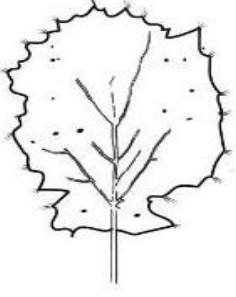
S3: The reproductive stage is achieved after the dormancy breaks in the month of March and stays till May during which the plant flowers and the fruits (silique) set in.

S4: The duration from setting of silique till their harvestable maturity is stage S4.




S5: The last stage of the plant life when seeds have been harvested and threshed out of silique.

IX. Explanations on the table of characteristics



Characteristic 1. Seedling: Leaf pubescence

	
	
1	9
Absent	Present
Seedling leaf pubescence	











Characteristic 2. Leaf: Colour

		
1	2	3
Light green	Dark green	Yellow-green
Leaf colour		







Characteristic 3. Leaf: Midrib colour

	
1	2
Green	Purple
Leaf midrib colour	

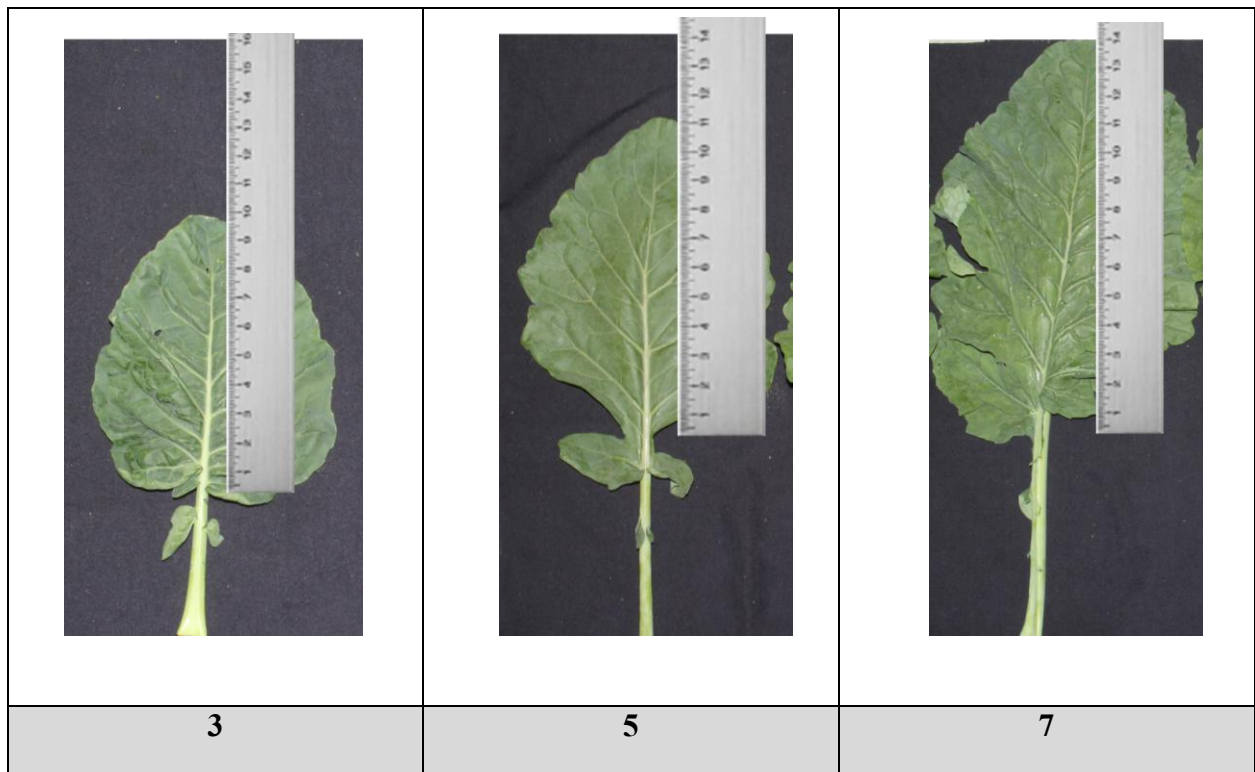
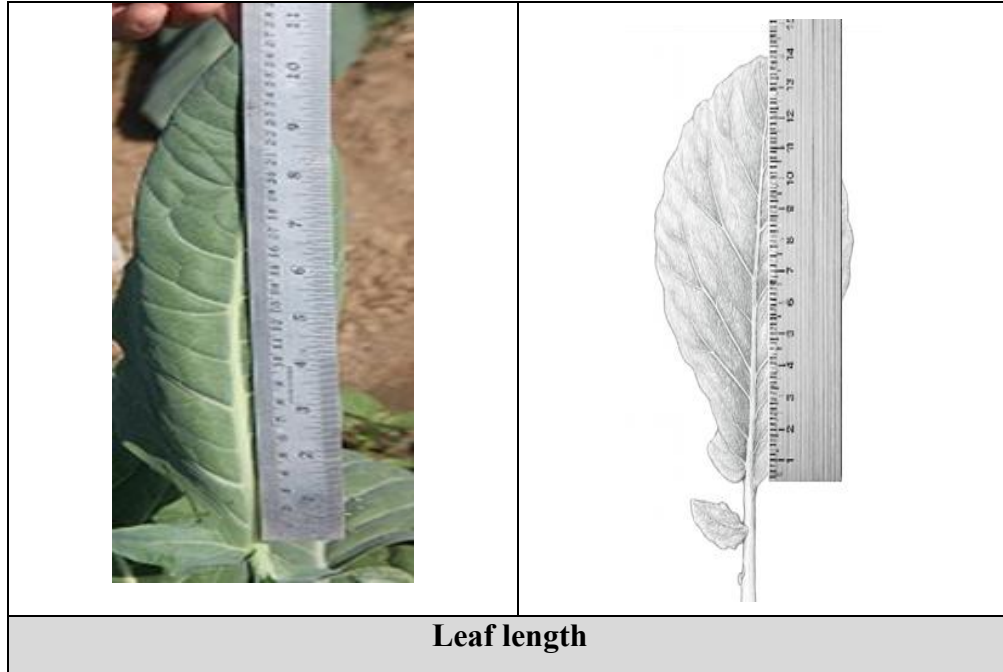
Characteristic 4. Leaf: Shape

				
				
1	2	3	4	5
Elliptic	Lanceolate	Obovate	Orbicular	Lobed
Leaf shape				

Characteristic 5. Leaf: Apex shape


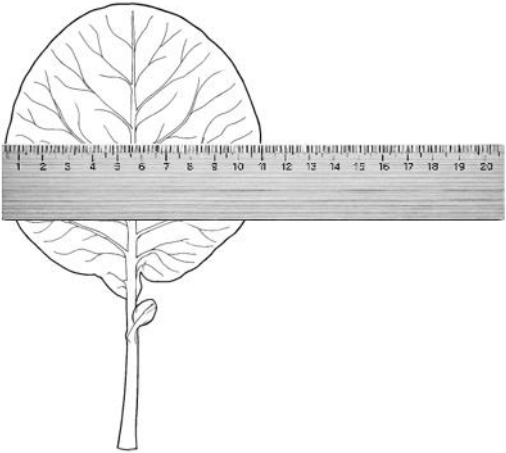
		
		
1	2	3
Round	Obtuse	Acute
Leaf apex shape		




Characteristic 6. Leaf: Length (cm)



Short	Medium	Long
Leaf length		





Characteristic 7. Leaf: Width (cm)

	
Leaf width	





		
3	5	7
Narrow	Medium	Broad

Leaf width



Characteristic 8. Leaf: Incisions

	
	
1	9
Absent	Present
Leaf incisions	




Characteristic 9. Leaf: Depth of incisions

			
1	2	3	4
Absent	Shallow	Medium	Deep
Depth of incisions			




Characteristic 10. Leaf: Waviness

	
1	9
Absent	Present
Leaf waviness	





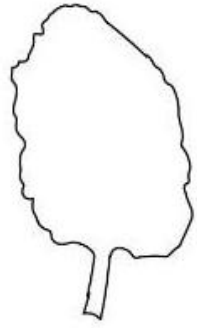

Characteristic 11. Leaf: Margin

		
1	2	3
Entire	Crenate	Lobed
Leaf margin		



Characteristic 12. Leaf: Puckering

		
1	3	5
Absent	Moderate	Dense
Leaf puckering		

Characteristic 13. Leaf: Re-curvedness of margin

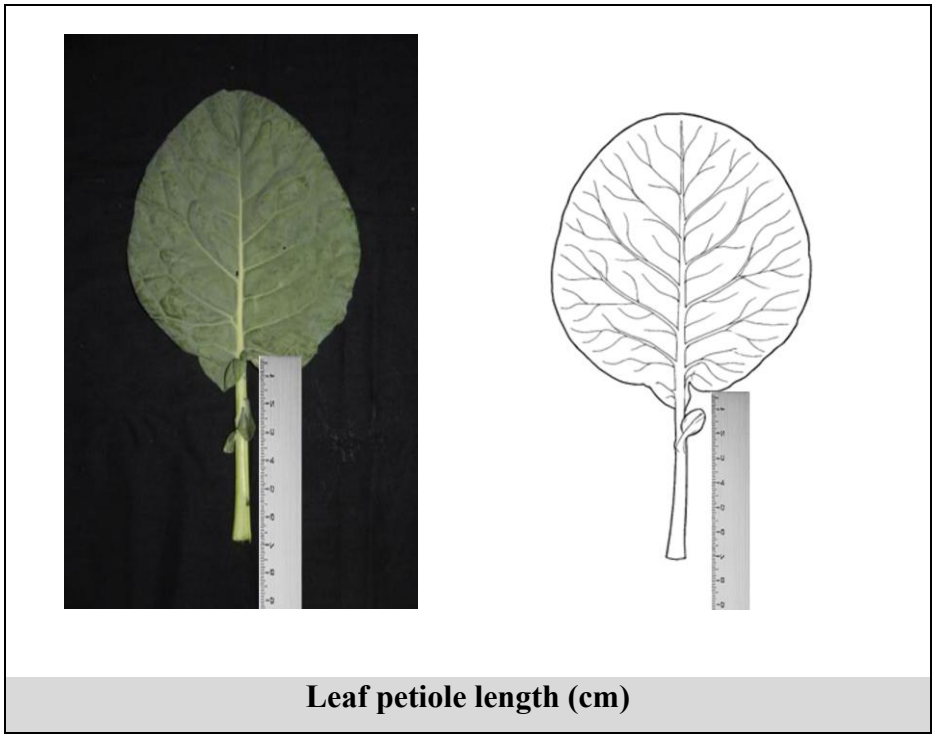
		
		
Weak	Medium	Strong
Recurvature of leaf margin		




Characteristic 14. Leaf: Presence of lobes

	
1	9
Absent	Present

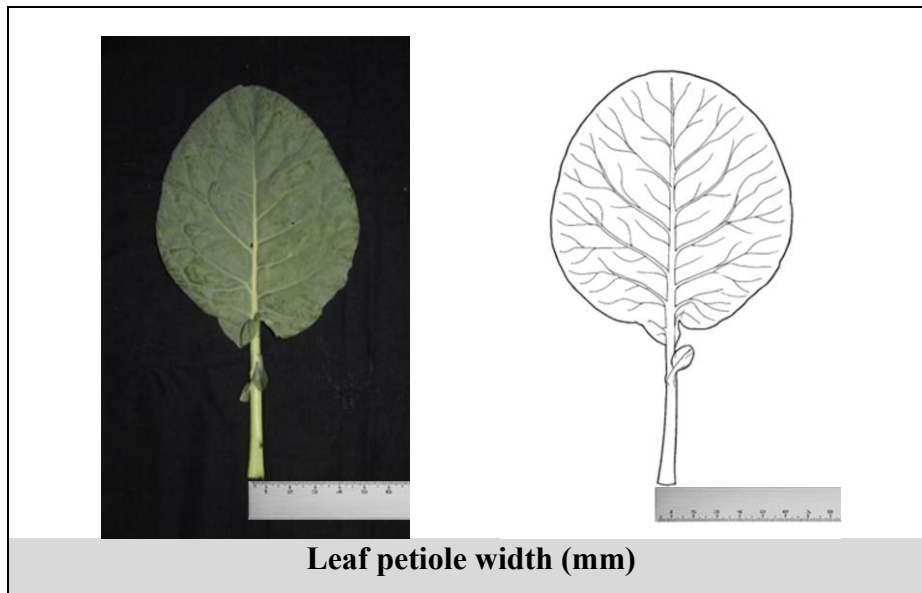
Presence of leaf lobes




Characteristic 15. Leaf: Petiole length (cm)





		
3	5	7
Short	Medium	Long
Leaf petiole length		

Characteristic 16. Leaf: Petiole width (mm)

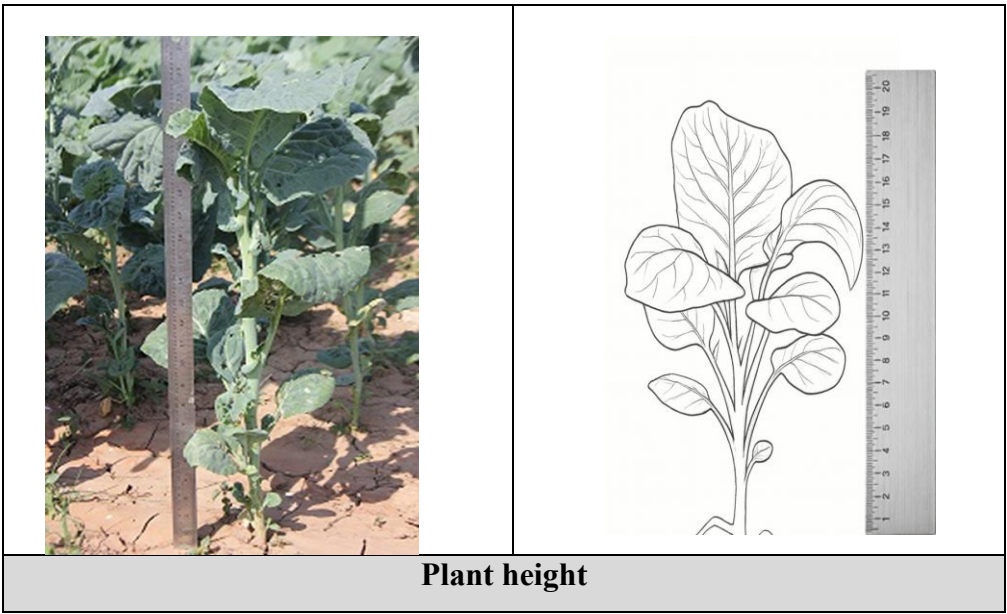


		
3	5	7
Narrow	Medium	Broad
Leaf petiole width		

Characteristic 17. Plant: Stem colour

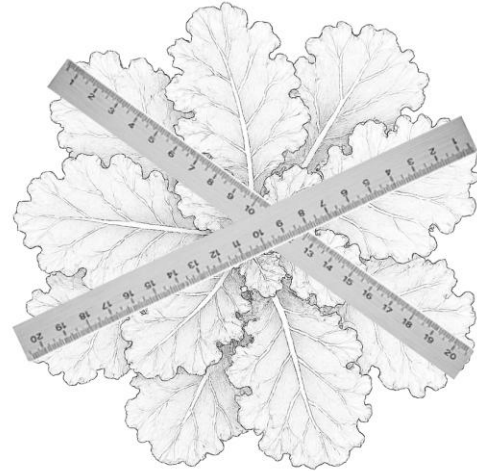
	
1	2
Green	Purple
Plant stem colour	

Characteristic 19. Plant: Height (cm)



3	5	7
Short	Medium	Tall
Plant height		

Characteristic 20. Plant: Canopy diameter (cm)



Plant canopy diameter



3



5



7







Small

Medium





Large

Plant canopy diameter

Characteristic 21. Plant: Growth habit

		
		
<p style="text-align: center;">1</p>	<p style="text-align: center;">2</p>	<p style="text-align: center;">3</p>
<p style="text-align: center;">Erect</p>	<p style="text-align: center;">Semi-erect</p>	<p style="text-align: center;">Prostrate</p>
<p style="text-align: center;">Plant growth habit</p>		

Characteristic 22. Plant: Main stem branching

	
	
1	9
Absent	Present
Main stem branching	

Characteristic 23. Inflorescence: Axis growth



1






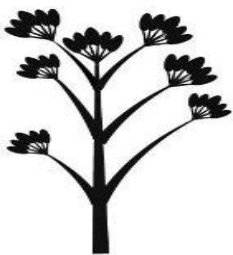
9

Condensed




Elongate

Inflorescence axis growth



Characteristic 24. Floral stalk: Branching

		
		
3	5	7
Sparse	Intermediate	Profuse
Floral stalk branching		




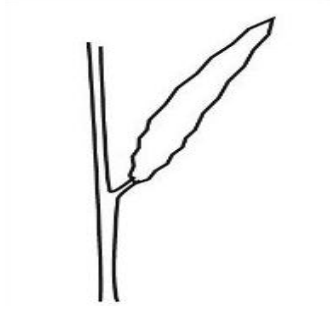
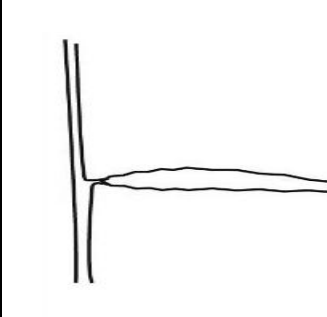
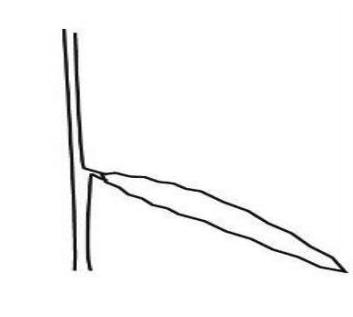
Characteristic 25. Floral bud: Colour

		
1	2	3
Green	Yellow	Purple
Floral bud colour		

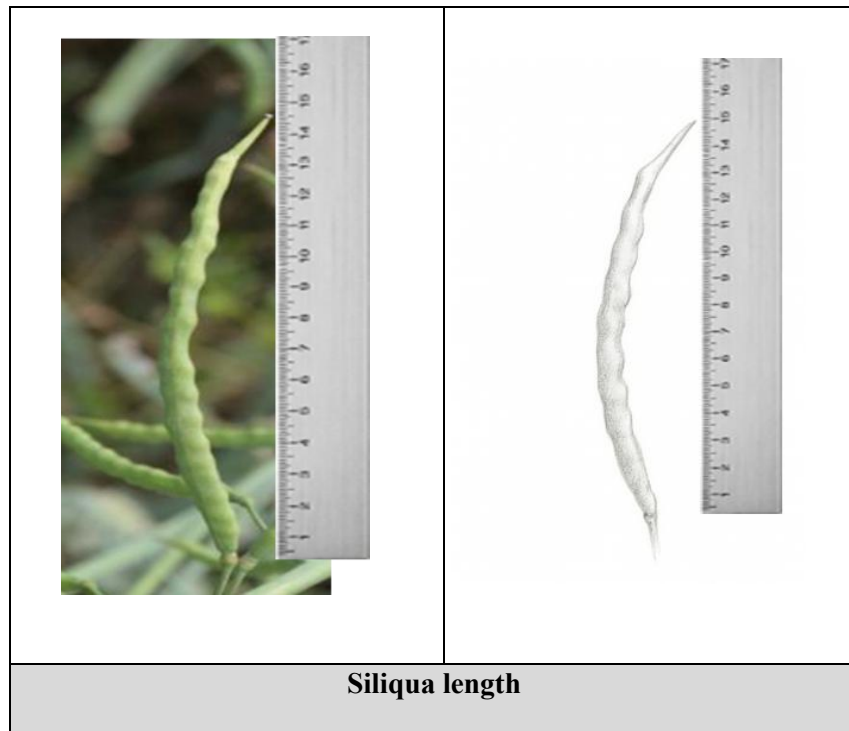
Characteristic 26. Flower: Stalk colour

	
1	2
Green	Purple
Flower stalk colour	

Characteristic 27. Siliqua: Attitude





		
		
3	5	7
Upright	Horizontal	Drooping
Siliqua attitude		

Characteristic 28. Siliqua: Length



1	2	3
Short	Intermediate	Long
Siliqua length		

Characteristic 29. Siliqua: Colour before drying

			
1	2	3	4
Green	Yellow	Violet	Purple
Siliqua colour before drying			

Characteristic 32. Seed: Colour

		
1	2	3
Brown	Greyed orange	Black
Seed colour		

X. Working Group details

The Test Guidelines developed by the National Core Committee in consultation with the Director, ICAR-Central Institute of Temperate Horticulture, Srinagar Nodal Officer, DUS Testing centre and the Task Force (05/2024) constituted by the PPV & FR Authority.

Technical Advisory Committee of Task Force

1	Dr. T A More, Former VC, MPKV, Rahuri	Chairman
2	Dr. Anil Khar, Head and PS, IARI-RS, Pune	Member
3	Dr. E Roshini Nayar, PS (Rtd), ICAR-NBPGR	Member
4	Dr. Geetika Malik, Scientist/PI, ICAR-CITH, Srinagar	PI of DUS Project
5	Dr. A K Singh, Plant Variety Examiner, PPVFRA, New Delhi	Member Secretary

XI. Name of DUS Test Centre(s): ICAR-Central Institute of Temperate Horticulture, Old Air Field, Rangreth, Srinagar 191132.