

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

On

**Lettuce
(*Lactuca sativa* L.)**

(Valid from)



**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	
I	Subject
II	Seed material required
III	Conduct of tests
IV	Methods and observations
V	Grouping characteristics
VI	Characteristics and symbols
VII	Table of characteristics
VIII	Explanation for the Table of characteristics
IX	Working group details
X	Name of DUS test centers

Draft DUS Guidelines

(Valid from)

Lettuce (*Lactuca sativa* L.)

I. Subject

These DUS test guidelines shall apply to all varieties, hybrids and parental lines of **Lettuce (*Lactuca sativa* L.)**

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	:	10 g in case of typical varieties and hybrids, and 5 g for each of the parental lines of the hybrid (equal weighing 10 packets in a single lot for variety and equal weighing 5 packets in a single lot for parental lines of a hybrid)
Extant (Variety of Common Knowledge) and Farmers category	:	5 g in case of typical variety or hybrid, and 5 g for each of the parental lines of hybrid (equal weighing 5 packets in single lot)
Extant (Notified) category	:	2 g in case of typical variety or hybrid, and 2 g for each of the parental lines of the hybrid (equal weighing 2 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 prior to the date of submission. They shall possess the highest genetic purity, uniformity and phytosanitary standards.
3. The seeds supplied should be visibly healthy, not lacking in vigour or affected by any important pest or disease. In case the seed is produced outside of the country, a phytosanitary certificate along with a 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 the maintainer parent of male sterility, if any, along with the protocol for hybrid seed production, also have to be supplied as per the category above, including seeds of the hybrid.
5. The designated DUS testing center shall maintain the variety during the testing period. After registration, it may be transferred to the reference variety collection, which 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 the 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 candidate variety's ecosystem.
2. The field test shall be carried out under conditions favouring normal growth and expression of all test characteristics. The size of the plot should be such that plants or parts of plants could be removed for measurement and observation without prejudicing the other observations 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 share similar environmental conditions of the test location.

3. The test plot design shall be as follows:

Experimental Design	:	Randomized Block Design (RBD)
No. of replications	:	03
Date of sowing	:	Mid-October
Date of Transplanting	:	Mid-November
Crop spacing	:	45 cm x 30 cm
Crop management practices	:	As per standard procedure
Plot size	:	9.25 sq. me (3.6 m x 2.5 m)
No. of plants per replication	:	60 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 second whorl from inner side.
6. The head characters except specified should be recorded at fully developed compact stage.
7. For the assessment of colour characteristics, the latest Royal Horticultural Society (RHS) colour chart shall be used.
8. Stage of recording observations on specific characteristics shall be as follows:

	Description	Code
a)	Seedlings	10
b)	Active vegetative growth (60 days after transplanting)	20
c)	50% plants in harvesting maturity	40
f)	Flowering stage (50% plants)	60
g)	Harvested and dried seeds	80

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: -**

Sl	Trait	Character No.	Stage of observation
a)	Plant: Type	Character-02	40
b)	Leaf: Anthocyanin colouration	Character-12	20
c)	Leaf: shape	Character-07	20
d)	Leaf: shape of apex	Character-08	20
e)	Time of harvesting	Character-	40

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 (Annexure II and Annexure III).

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	Reference varieties (public sector)	Stages of observation	Type of Assessment
1.	Seedling: Anthocyanin colouration of young leaves (*)	Absent	1	Chinese Yellow, Solan Kriti, Phule Padma, Great Lakes, Iceberg.	10	VG
		Present	9	Harit Baingani, Red Romaine,		
2.	Plant: Type (*) (+)	Romaine	1	Harit Baingani, Red Romaine.	40	VG
		Butter head	3	EC1177325		
		Crisphead	5	Iceberg, Great Lakes		
		Leafy	7	Chinese Yellow, Solan Kriti, Phule Padma		
3.	Plant Frame: diameter (*)	Small (<30 cm)	1	-	40	MS
		Medium (30.1-45 cm)	3	Solan Kriti, Tango		
		Large (>45 cm)	5	Phule Padma, Chinese Yellow		
4.	Plant: degree of overlapping of the upper part of leaves	Absent	1	DL-Sel-90	40	VG
		Weak	5	Chinese Yellow, Phule Padma		
		Strong	7	Great Lakes, Iceberg		

	(*) (+)					
5.	Plant: number of leaves	Few (<20)	3	Iceberg	20	MS
		Medium (20.1-30)	5	Great Lakes, Phule Padma		
		Many (>30)	7	Chinese Yellow		
6.	Plant: attitude	Erect	1	Harit Baingani	20	VG
		Semi-erect	3	Great Lakes, Iceberg, Chinese Yellow, Solan Kriti, Phule Padma		
		(*)(+) Horizontal	5	-		
7.	Leaf: shape	Narrow elliptic	1	Phule Padma, Red Romaine	40	VG
		Broad obtrullate	3	Red Rose		
		Obovate	5	Great Lakes, Iceberg, Almao-1		
		(*)(+) Oblanceolate	7	Chinese Yellow, Solan Kriti		
		Lobed	9	-		
8.	Leaf: Length	Short (<20 cm)	1	Alamo-1, Tango	40	MS
		Medium (20- 25 cm)	3	Great Lakes, Solan Kriti, Phule Padma		
		(*) Long (>25 cm)	5	-		
9.	Leaf: Width	Narrow (<15 cm)	1	Red Salad	40	MS
		Medium (15-18 cm)	3	Chinese Yellow, Harit Baingani, Solan Kriti,		
		(*) Broad (>18 cm)	5	Great Lakes		
10.	Leaf: Anthocyanin colouration	Absent	1	Chinese Yellow, Great Lakes, Iceberg	40	VG
		Medium	5	Harit Baingani, Red Romaine		
		(*)(+) Strong	7	Red Rose		
11.	Leaf: hue of anthocyanin coloration	Absent	1	Chinese Yellow, Iceberg,	40	VG
		Red	3	-		
		Brown	5	-		
		(*) Purple	7	Red Salad, Red Romaine		
12.	Leaf: area covered by anthocyanin coloration	Absent	1	Chinese Yellow, Great Lakes, Solan Kriti, Phule Padma, Ice berg, Almao-1	40	VG
		(*)(+) Medium	3	Red Romaine.		
		Large	5	Harit Baingani, Red Salad, Red Rose.		
13.	Leaf: colour	Green	1	-	40	VG

		Yellowish Green	2	Chinese Yellow, Great Lakes, Solan Kriti, Phule Padma		
		Greyish Green	3	-		
	(*)(+)	Greyish Purple	4	Red Salad, Red Romaine		
		Purple	5	-		
14.	Leaf: Intensity of green colour	Light	1	Chinese Yellow	40	VG
	(*)	Medium	3	Great Lakes, Solan Kriti		
		Dark	5	Iceberg		
15.	Leaf: Glossiness of the upper side	Weak	3	-	40	VG
	(*)	Medium	5	Great Lakes, Solan Kriti, Phule Padma		
		Strong	7	Harit Baingani		
		Very strong	9	Red Salad, Red Romaine, Red Rose		
16.	Leaf: blistering	Weak	1	Harit Baingani	40	VG
	(*)(+)	Medium	3	Chinese yellow, Great Lakes		
		Strong	5	Red Romaine		
17.	Leaf: undulation of the margin	Absent or very weak	1	Chinese Yellow, Harit Baingani, Solan Kriti,	40	VG/VS
	(*)(+)	Weak	3	Red Salad, Great Lakes, Phule Padma		
		Medium	5	Iceberg, Tango		
		Strong	7	Alamo-1		
18.	Leaf: type of incisions of the margin	Entire or Absent	1	Harit Baingani, Solan Kriti, Chinese Yellow.	40	VG
	(*)(+)	Crenate	2	-		
		Regularly dentate	3	Phule Padma, Red Salad.		
		Irregularly dentate	4	Iceberg, Tango, Great Lakes.		
		Bidentate	5	Red Rose, Alamo-1		
		Tridentate	6	-		
19.	Leaf: depth of incisions of margin	Absent or very shallow	1	Chinese Yellow, Harit Baingani, Solan Kriti, Red Romaine, Tango	40	VG
	(*)(+)	Shallow	3	Phule Padma		
		Medium	5	Alamo-1, Iceberg		
		Deep	7	Alamo-1, Red Rose		
20.	Leaf: density of incisions of margin	Absent		Chinese yellow	40	VG
		Medium	3	Red Salad, Great Lakes, Phule Padma,		

		Dense	5	Iceberg, Alamo-1, Red Rose		
21.	Head: shape in longitudinal section	Narrow elliptic	1	Harit Baingani, Red Romaine.	40	VG
		Broad elliptic	2	-		
		Circular	3	Great Lakes, Iceberg, Alamo-1.		
		Narrow oblate	4	-		
	(*) (+)					
22.	Stem: colour	Whitish green	3	Chinese Yellow, Solan Kriti.	60	VG
		Green	5	Phule Padma.		
		Purple	7	Red Salad, Harit Baingani, Red Romaine.		
	(*) (+)					
23.	Plant: Time of Marketable maturity	Early (<75days)	3	Harit Baingani, Red Romaine, Red Rose.	60	MG
		Medium (75-100 days)	5	Chinese Yellow, Solan Kriti, Phule Padma, Tango, Red Salad.		
		Late (>100 days)	7	Great Lakes, Alamo-1, Iceberg.		
	(*)					
24.	Seed: colour	White	1	Chinese Yellow, Great Lakes, Solan Kriti, Phule Padma, Red Romaine, Iceberg, Alamo-1,	80	VG
		Black	3	Red Salad, Harit Baingani,		
	(*)					

VIII. Explanations covering several characteristics

Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

(a) Plant, head and stem: Observations should be made at harvest maturity. For varieties with degree of overlapping of upper part of leaves absent or weak observations should be made just before deterioration and before bolting.

(b) Leaf: For varieties with degree of overlapping of upper part of leaves medium or strong observations should be made on the largest outer leaves, at harvest maturity. For varieties with degree of overlapping of upper part of leaves absent or weak observations should be made on the largest leaves, just before deterioration and before bolting.

IX. Working Group details:

The test guidelines developed by the task force constituted by the PPV & FR Authority for **Lettuce (*Lactuca sativa* L.)** with consultation by Nodal officer, ICAR-IARI, New Delhi. The Technical inputs also provided by the PPV & FR Authority.

1.	Dr Pritam Kalia Former Head and Professor Division of Vegetable Science ICAR-Indian Agricultural Research Institute New delhi-110012	Chairman
2.	Dr S. R. Sharma Former Head ICAR-Indian Agricultural Research Institute, Regional Station, Katrain, Kullu, HP-175129	Member
3.	Dr Chithra Devi Pandey Principal Scientist ICAR-National Bureau of Plant Genetic Resources New Delhi-110012	Member
4.	Dr Shyam Sundar Dey Principal Scientist Division of Vegetable Science ICAR-Indian Agricultural Research Institute New Delhi-110012	Member/PI
5.	Dr Uma Kant Dubey Deputy Registrar PPV&FRA, New Delhi-110012	Member Secretary

X. Name of DUS test centers

Lead Centre
ICAR-Indian Agricultural Research Institute, New Delhi-110012
Cooperating Centre
ICAR-Indian Agricultural Research Institute, Regional Station, Katrain, Himachal Pradesh, 175129

Nodal Officer:

Dr Shyam Sundar Dey, Principal Scientist, Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi-110012

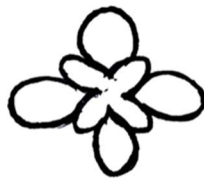
Annexure-II

Diagrammatic explanation for individual characteristics

Characteristic 4: Plant: degree of overlapping of the upper part of leaves



Absent



Weak



Strong

Characteristic 6: Leaf: attitude



**1
Erect**



**3
semi-erect**

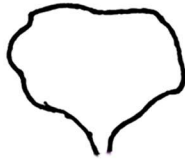


**5
horizontal**

Characteristic 7: Leaf: shape



Narrow elliptic



Broad obtrullate



Obovate



Oblanceolate

Characteristic 17: Leaf: undulation of the margin



Absent or very weak



Weak



Medium



Strong



Very strong

Characteristic 18: Leaf: type of incisions of the margin



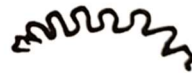
Absent



Shallow



Medium

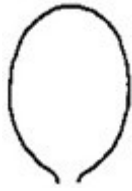


Deep

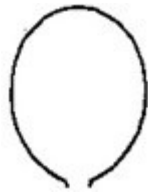


Very deep

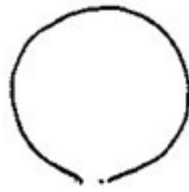
Characteristic 21: Head:shape in longitudinal section



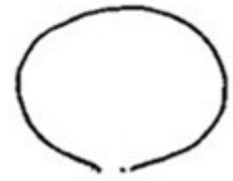
Narrow elliptic



Broad elliptic



Circular



Narrow oblate

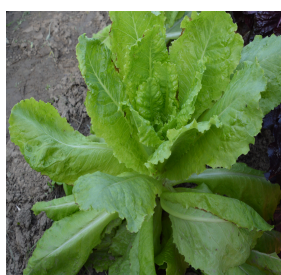
Annexure-III

Coloured photographs of important traits of Lettuce for DUS characterization

Characteristic 2: Plant: type



Characteristic 4: Plant: degree of overlapping of the upper part of leaves



Absent



Weak



Strong

Characteristic 6: Leaf: attitude



Erect



Semi-erect



Horizontal

Characteristics 7: Leaf: shape



Narrow elliptic



Broad obtrullate



Obvate



Oblanceolate

Characteristics 10: Leaf: Anthocyanin colouration



Absent

Medium

Strong

Characteristics 12: Leaf: area covered by anthocyanin of colouration



Absent

Medium

Large

Characteristic 13: Leaf: colour



Yellowish green



Grayish purple

Characteristic 15: Leaf: Glossiness of the upper side



Weak



Medium



Strong



Very strong

Characteristic 16: Leaf: blistering



Weak

Medium

Strong

Characteristic 17: Leaf: undulation of the margin



Absent or very weak



Weak



Medium



Strong



Very strong

Characteristics 18: Leaf: type of incisions of margin



Absent



Regularly dentate



Irregularly dentate



Bidentate



Tridentate

Characteristics 19: Leaf: depth of incisions of margin



Absent



Shallow



Medium



Deep



Very deep

Characteristic 21: Head:shape in longitudinal section



Narrow elliptic



Circular

Characteristic 22: Stem:colour



Whitish green



Light green



greenish purple