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

On

Barley

(***Hordeum vulgare*** **L.**)

Protection of Plant Varieties and Farmers Rights' Authority
(PPV & FRA)
Government of India

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I Subject

These test guidelines shall apply to all varieties, hybrids and parental lines of Barley (*Hordeum vulgare* L.*)*

II Material required

1. The Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) shall decide where and in what quantity and quality of the seed material are required for testing a variety denomination applied for registration under the Protection of Plant Varieties and Farmers' Rights Authority (PPV & FR Act), 2001. Applicants submitting such seed material from a country other than India shall make sure that all customs and quarantine requirements stipulated under relevant national legislation are complied with. The minimum quantity of seed to be provided by the applicant shall be 1500 grams in the case of the candidate variety or hybrid and 1000 grams for each of the parental lines of the hybrid. Each of these seed lots shall be packed and sealed in ten equal weighing packets and submitted in one lot.
2. At least 100 spikes, each representing the normal spike size and drawn from the main tiller of the candidate variety shall be submitted. The spikes shall be individually packed and submitted along with the said seed lot.
3. The seed and spikes submitted shall have at least 95% germination, 98% physical purity, highest genetic purity, uniformity, sanitary and phytosanitary standard. In addition the moisture content of the seed shall not exceed 8% to meet the safe storage requirement. The applicant shall submit along with the seed a certified data on germination test made not more than one month prior to the date of submission.
4. The seed material shall not be subjected to chemical and biophysical treatment.

III Conduct of tests

1. The minimum duration of the DUS tests for the new varieties shall normally be at least two independent similar growing seasons.

2. The test shall normally be conducted at least at two locations. If any essential characteristic of the candidate variety is not expressed for visual observation at these locations, the variety shall be considered for further examination at another appropriate test site or under special test protocol on expressed request of the applicant.

3. The field tests shall be carried out under conditions favoring normal growth and expression of all test characteristics. The size of the plots shall be such that plants or parts of plants could be removed for measurement and observation without prejudicing the other to the observations on the standing plants until the end of the growing period. Each test shall include about 500 plants, in the plot size and planting space specified below across three replications. Separate plots for observation and for measuring 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.

4. Test plot design

Number of rows	:	4
Row length	:	4 m
Row to row distance	:	30 cm
Plant to plant distance	;	10 cm
Expected plants/replication	:	160
Number of replications	:	3

5.Observations should not be recorded on plants in border rows

6.Additional test protocols for special test shall be established by the PPV&FR Authority.

IV Methods and observations

1. The characteristics described in the table of characteristics shall be used for the testing of varieties, inbred lines and hybrids for their DUS
2. For the assessment of distinctiveness and stability observation shall be made on 30 plants or parts of 30 plants, which shall be equally divided among 3 replications (10 plants per replication).
3. For the assessment of uniformity of characteristics on the plot as a whole, this shall be done on simple visual observation of a group of plants or parts of plant. During such observation the entry shall be deemed uniform when the number of aberrant or odd plants or parts of plant shall not be exceeding 2 in 500.
4. For the assessment of uniformity of characteristics on single spike-rows, plants or parts of plant shall be visually observed on all individual spike-rows, plants or parts of plants. A spike-row having at least one aberrant or odd plant or parts of plant is dealt as an aberrant row. A variety shall be deemed uniform when the number of such aberrant spike-rows shall not exceed 2 in 100.
5. For the assessment of color characteristics, the latest Royal Horticultural Society (RHS) color chart shall be used.

V. Grouping of varieties

1. The candidate varieties for DUS testing shall be divided into groups to facilitate the assessment of Distinctiveness. Characteristics, which are 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. The following characteristics are proposed to be used for grouping barley varieties:
   a) Stem: Basal pigmentation (Characteristic 2)
   b) Auricle: Anthocyanin pigmentation (Characteristic 3)
   c) Spike emergence (Characteristic 7)
   d) Spike type (row number) (Characteristic 8)
   e) Plant height (Characteristic 20)
   f) Spike density (Characteristic 25)
   g) Grain hullness (Characteristic 26)
   h) Grain: colour (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 (Section VII) shall be used.

2. Scale 1 to 9 is used to describe the state of each character for the purpose of digital data processing.

3. The optimum stage for taking the observation of each characteristic during the plant growth and development is indicated by a decimal code. The Zadoks system being the most universally accepted is described here. It is applicable to any small grain, and its stages are easy to identify in the field. The Zadoks system is a two-digit code where the first digit refers to the principal stage of development beginning with germination and ending with kernel ripening. The second digit (between 0 and 9) subdivides each principal growth stage. The relevant growth corresponding to the decimal code number are described in section VII, column 5.

4. Legend :

(*) Characteristics that should be observed during every growing period on all varieties and should always be included in the description of the variety, except when the state of expression of any of these characters is rendered impossible by a preceding phenological characteristic or by the environmental conditions of the testing region. Under such exceptional situation, adequate explanation should be provided.

(+) See Explanations on the table of characteristics in Chapter VIII.

5. Type of assessment of characteristics indicated in the table 2 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 plants or parts of plants

VG: Visual assessment by a single observation on a group of plants or parts of plants

VS: Visual assessment by observations of individual plants or parts of plants

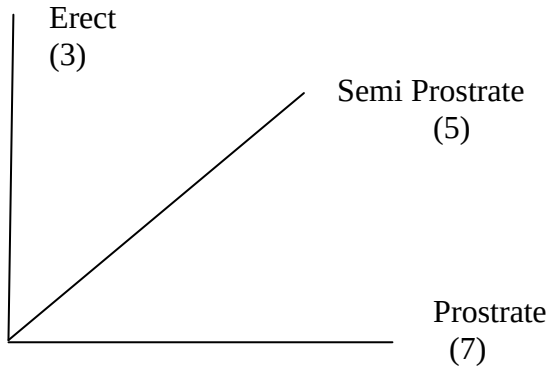
VII Table of characteristics

S.NO (1)	Characteristics (2)	State (3)	Note (4)	Stage of observation (5)	Example variety (6)	Type of assessment (7)
1. * (+)	Growth habit	Erect Semi-prostrate Prostrate	3 5 7	23-25	Amber Alfa93 ---	VG
2. *	Stem: Basal pigmentation	Absent Present	1 9	25-33	Amber Alfa93	VG
3. *	Auricle (Flag leaf): Anthocyanin Pigmentation	Absent Present	1 9	49-59	Amber Alfa93	VG
4. *	Upper node Pigmentation	Absent Present	1 9	49-59	Amber Alfa93	VG
5. * (+)	Flag leaf attitude	Erect Semi-erect Drooping	1 5 9	51-59	Amber BCU73 Alfa93	VG
6. *	Flag Leaf: Waxiness of sheath	Absent Present	1 9	51-59	Ratna Alfa93	VG
7. *	Spike emergence	Very early (<65 days) Early (65-75 days) Medium (76-86days) Late (87-96 days) Very late (> 96days)	1 3 5 7 9	51-59	----- BCU73 Amber Dolma Alfa93	MG
8. * (+)	Spike type	Two-row Six-row	3 7	59-69	Alfa93 Amber	VG
9. (+)	Lateral florets (Two-row barley)	Rudimentary Developed	1 9	59-69	DWRB73 Alfa93	VG
10. *	Spike: Waxiness	Absent Present	1 9	59-85	BCU73 Alfa93	VG
11.	Spike: colour	Pale green Green Dark green	1 2 3	69-77	DWR28 Alfa93 DWRUB64	VG
12. *	Spike: attitude	Erect Semi-erect Drooping	3 5 7	69-77	Amber BHS169 Alfa93	VG
13.	Awn: roughness	Smooth Rough	3 7	69-77	BH393 Alfa93	VG
14	Flag leaf length	Short (<10cm) Medium (10-14 cm) Long (> 14 cm)	3 5 7	71-85	Alfa93 Amber Ratna	MS
15.	Flag leaf breadth	Narrow (<1.0 cm) Medium (1.0-1.5 cm) Wide (>1.5 cm)	3 5 7	71-85	Alfa93 Amber BH75	MS
16.	Awn: Tip pigmentation	Absent Present	1 9	73-87	Amber BH393	VG
17.	Spike: basal sterility	Absent Present	1 9	73-89	Alfa93 RD2715	VS
18.	Lemma: pigmentation	Absent Nerve pigmented Present	3 5 7	75-87	Dolma Alfa93 DWRB91	VG
19.	Spike: length	Small (< 7cm) Medium (7.1-10cm) Long (>10cm)	3 5 7	75-89	Ratna BH75 Alfa93	MS
20. *	Plant: height	Very short (< 75.0 cm) Short (75.1-85.0 cm) Medium (85.1-95.0 cm) Tall (95.1-105.0 cm) Very tall (> 105.0 cm)	1 3 5 7 9	75-89	---- BCU73 Alfa93 BHS169 Amber, Jyoti	MG

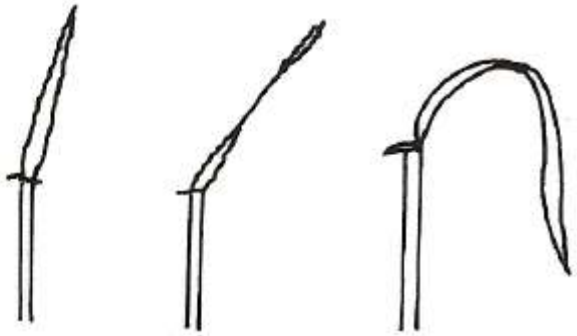
21.	Peduncle: length	Short (<22.0 cm) Medium (22.0 -27.0 cm) Long (> 27.0 cm)	3 5 7	75-89	Alfa93 Amber Dolma	MS
22.	Awns	Absent Present	1 9	83-87	---- Alfa93	VG
23. * (+)	Awns: type	Hooded Awnletted Normal	1 3 5	83-87	--- --- Alfa93	VG
24.	Awn: length	Short (< 8.0 cm) Medium (8.0-11.0 cm) Long (>11.0 cm)	3 5 7	83-87	BH75 BHS169 Alfa93	MS
25. * (+)	Spike: density	Lax Intermediate Dense	3 5 7	83-89	Azad, Jyoti Alfa93 Ratna	VG
26.	Grain: hullness	Naked (hulless) Covered (Hulled)	1 9	87-92	Dolma Alfa93	VS
27. *	Grain: color	White Yellow Purple Black	1 2 3 4	92	Dolma Alfa93 Bilara2, Jyoti ----	VG
28. * (+)	Grain: shape	Oval Oblong Elliptical	1 5 7	92	Alfa93 BHS169 Amber	VG
29. *	Grain: size (1000 grain weight)	Small (<30g) Medium (30-40g) Large (41-50g) Very large (>50g)	1 3 5 7	92	Dolma Alfa93 Amber BCU73	MS
30.	Grain: surface	Smooth Wrinkled	1 9	92	Amber JB58	VG
31. (+)	Rachilla hairs	Rudimentary Prominent	1 9	92	Alfa93 BHS46	VS
32. * (+)	Grain: Crease width	Narrow Intermediate Wide	3 5 7	92	BHS169 Alfa93 -----	VS

VIII. Explanations on the Table of characteristics.

Characteristic 1. Growth habit

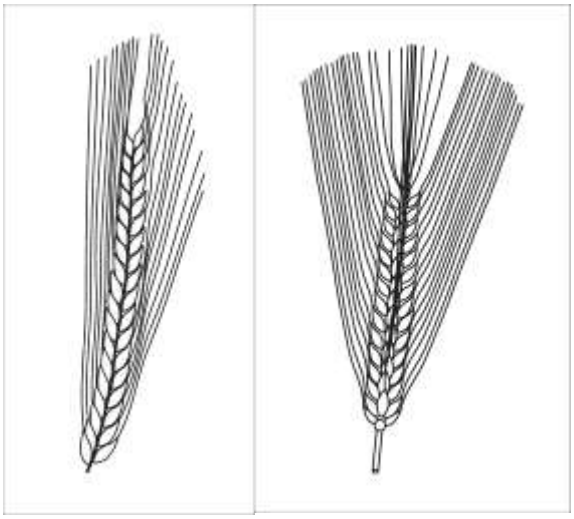


Characteristics 5: Flag leaf attitude



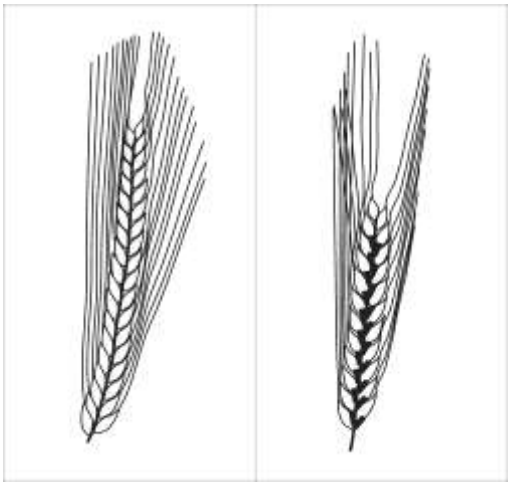
Erect (1) Semi erect (5) Drooping (9)

Characteristics 8: Spike type



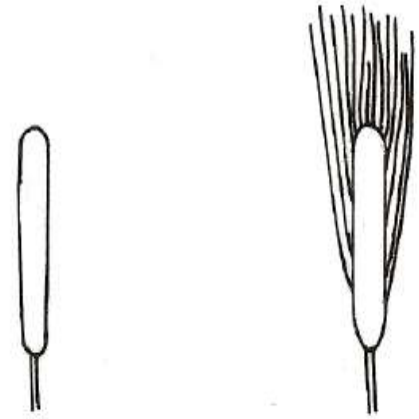
Two-row (3) Six-row (7)

Characteristics 9: Lateral florets (Two-row barley)



Rudimentary (1) Developed (9)

Characteristics 22: Awns



Absent (1) Present (9)

Characteristics 23: Awns: Type

Hooded (1) Awnletted (3) Normal (5)

Characteristics 25: Spike: density

Lax
(3)

Intermediate
(5)

Dense
(7)

Characteristics 28: Grain: Shape

Oval
(1)

Oblong
(5)

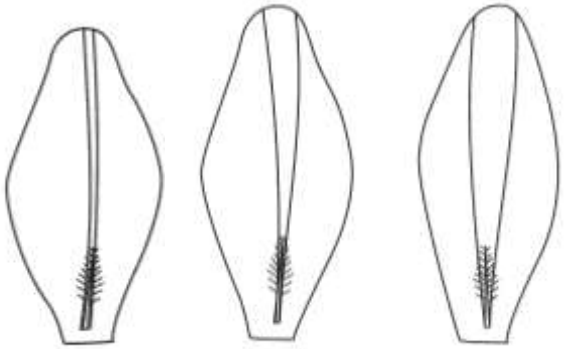
Elliptical
(7)

Characteristics 31: Rachilla hairs

Rudimentary
(1)

Prominent
(9)

Characteristics 32: Grain crease width



Narrow (3) Intermediate (5) Wide (7)

IX. Biochemical characters (Additional character)

1.	Hordein Profile	Electrophoretic profile of the candidate variety to be generated for A, B, C and D sub units of the hordein storage protein using the mature grains harvested from the experiments.

Procedure for estimation of hordein profile:

Hordeins are the major storage proteins of barley endosperm. They are extremely heterogeneous in composition in the different barley cultivars, allowing the differentiation of genotypes by their protein electrophoretic patterns. In general, barley hordeins are divided into A, B, C and D groups on the basis of the molecular weight differences due to their amino acid compositions. Since hordein composition is normally not affected by environmental factors (e.g. growing location, soil types, fertilization level, etc.), hordein electrophoretic composition could be used as genetic character for cultivar identification. By far, poly-acrylamide gel electrophoresis (PAGE) is the most common methods for the protein separation, characterization and varietal identification in barley (Laemmli, 1970).

Reference

Laemmli, U.K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227, 680–685.

X. Working group details

The test guidelines developed by the Project Director, Directorate of Wheat Research (ICAR), Karnal and Nodal officer DUS (Barley) testing centre DWR, Karnal and the Task Force (3/2011) constituted by the PPV&FR Authority. Technical input was also provided by Dr. S. R. Vishwakarma, NDUA&T, Faizabad; Sh. Dipal Roy Choudhury, Sh. D. S. Mishra, Dr. D. S. Pilania, Dr. Ravi Prakash and Dr. Manoj Srivastava (PPV&FRA).

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XI. DUS Test Centres

Nodal DUS centre	**Co-Nodal centre(s)**
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	Agriculture Research Station, (Swami Keshwanand Rajasthan Agriculture University), Durgapura, Jaipur, Rajasthan

Annexure-I System for growth stages in barley

Zadok's code		Description
Principal stage	**Secondary stage**	
0		Germination
	0	Dry kernel
	1	Start of imbibition (water absorption)
	5	Radical emerged
	7	Coleoptile emerged
	9	Leaf just at coleoptile tip
1		Seeding development
	0	First leaf through coleoptile
	1	First leaf at least 50% emerged
	2	Second leaf at least 50% emerged
	3	Third leaf at least 50% emerged
	4	Fourth leaf at least 50% emerged
	5	Fifth leaf at least 50% emerged
2		Tillering
	0	Main shoot only
	1	Main shoot plus 1 tiller visible
	2	Main shoot plus 2 tillers
	3	Main shoot plus 3 tillers
	4	Main shoot plus 4 tillers
	5	Main shoot plus 5 tillers
3		Stem elongation
	1	First node detectable
	2	Second node detectable
	3	Third node detectable
	7	Flag leaf just visible
	9	Flag leaf collar just visible
4		Boot
	1	Flag leaf sheath extending
	3	Boot just beginning to swell
	5	Boot swollen
	7	Flag leaf sheath opening
	9	First awns visible
5		Head emergence
	1	First spikelet of head just visible
	3	One-fourth of head emerged
	5	One-half of head emerged
	7	Three-fourths of head emerged
	9	Head emergence complete
6		Flowering (not readily visible in barley)
	1	Beginning of flowering
	5	Half of florets have flowered
	9	Flowering complete
7		Milk development in kernel
	1	Kernel watery ripe
	3	Early milk
	5	Medium milk
	7	Late milk
8		Dough development in kernel
	3	Early dough
	5	Soft dough
	7	Hard dough, head losing green color
	9	Approximate physiological maturity
9		Ripening
	1	Kernel hard (difficult to divide with thumbnail)
	2	Kernel cannot be dented by thumbnail, harvest ripe