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

On

Bread Wheat

(*Triticum aestivum* L.)

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

Government of India

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CONTENTS

I. Subject

These test guidelines shall apply to all varieties, hybrids and parental lines of Bread wheat (*Triticum aestivum* L.)

II. Seed 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 Variety and Farmers' Rights (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 legislations and regulations are complied with. The minimum quantity of the seed to be provided by the applicant shall be 3000 gram in the case of the candidate variety or hybrid and 1500 gram for each of the parental line of the hybrid. Each of these seed lots shall be packed and sealed in ten equal weighing packets and submitted in one lot. Wherever, individual spikes are to be supplied, such spikes shall be individually packed and submitted along with the said seed lot.

2. At least 100 ears, each representing the normal ear size and drawn from the main tiller of the candidate variety shall be submitted.

3. The seeds and ears submitted shall have at least 95 % germination, 98% physical purity, highest genetic purity, uniformity, sanitary and phyto-sanitary standards. In addition the moisture content of the seed shall not exceed 8 - 9% to meet the safe storage requirement. The applicant shall also 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 have been subjected to any chemical and bio-physical treatment.

III. Conduct of tests

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

2. The test shall normally be conducted at least at two test locations. If any essential characteristics of the candidate variety are 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 favouring 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 observations on the standing plants until the end of the growing period. Each test shall include about 1000 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	:	6
Row length	:	6 m
Row to row distance	:	30 cm
Plant to plant distance	:	10 cm
Expected plants / replication	:	360
Number of replications	:	3

5. Observations shall not be recorded on plants in border rows.
6. Additional test protocols for special purpose 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 observations 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 (visual assessment by observations of a number of individual panicle-rows, plants or parts of plants) the number of aberrant or odd plants or parts of plant shall not exceed 2 in 1000.
4. For the assessment of Uniformity of characteristics on single ear-rows, plants or parts of plant shall be visually observed on all individual ear-rows, plants or parts of plants. An ear-rows 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 ear-rows shall not exceed 3 in 100.
5. For the assessment of color characteristics, the latest Royal Horticultural Society (RHS) colour 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 distinctness. 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 bread wheat varieties:

    a) Flag leaf : Anthocynin coloration of auricle (Characteristic 4)

b) Time of ear emergence (Characteristic 7)

c) Plant length (Characteristic 14)

d) Awn or scurs : Presence (Characteristic 18)

e) Outer glume : Pubescence (Characteristic 23)

f) Ear : Colour (Characteristic 24)

g) Season type (Characteristic 37)

h) Grai n hardness (Characteristic 38)

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. Note (1 to 9) is used to describe the state of each character for the purpose of digital data processing.

3. Legend :

(*) Characteristics that shall be observed during every growing season on all varieties and shall 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 shall be provided.

(+) See Explanation on 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 or figure(s) for clarity and not the colour variation.

4. The optimum stage for the observation of each characteristic during the plant growth and development is indicated by a decimal code number in the sixth column of table of characteristics. The relevant growth stages corresponding to these decimal code numbers are described below:

Decimal Code for the Growth Stage

Growth Stage Code	Corresponding Growth Stage
Germination	
09	Leaf just at coleoptile tip
10	First leaf through coleoptile
11	First leaf unfolded
Tillering	
25	Main shoot and 5 tillers
26	Main shoot and 6 tillers
27	Main shoot and 7 tillers
28	Main shoot and 8 tillers
29	Main shoot and 9 tillers
Booting	
40	Early boot stage
41	Flag leaf sheath extending
43	Boots just visibly swollen
47	Flag leaf sheath opening
49	First awns visible [in awned forms only]
Inflorescence	
50-51	First spikelet of inflorescence just visible
52	1/4 of inflorescence emerged
Anthesis	
60-61	Beginning of anthesis
64-65	Anthesis half-way
68-69	Anthesis complete
Milk development	
73	Early milk
75	Medium milk

77	Late milk
	Dough development
83	Early dough
85	Soft dough
87	Hard dough
Ripening	
91	Caryopsis hard (difficult to divide by thumb-nail)
92	Caryopsis hard (can no longer be dented by thumb-nail)
93	Caryopsis loosening in daytime
94	Over-ripe, straw dead and collapsing

5. Type of assessment of characteristics indicated in column seven 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 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	Characteristics	States	Note	Example variety/line	Stage of observation	Type of assessment
1	2	3	4	5	6	7
1. (*) (+)	Coleoptile : Anthocyanin colouration	Absent Present	1 9	AKW381, AKW 1071 HD 2781, DWR195	09-11	VS
2. (*) (+)	Plant : Growth habit	Erect Semi-erect Intermediate Semi-prostrate Prostrate	1 3 5 7 9	K9644, HD 2842 AKW381, PBW222 HD1941, HW2004 VL616, ---	25-29	VG
3. (*)	Foliage : Colour	Pale green Green Dark green	1 5 9	HYB 633, C 306 CPAN 3004, AKW 381 HD2428, HP 1731	40-45	VG
4. (*)	Flag leaf : Anthocyanin colouration of auricles	Absent Medium Very strong	1 5 9	AKW1071, CPAN 3004 HD1949, HD2428 GW 89,HD2009	49-51	VG
5. (*)	Flag leaf : Hairs on auricle	Absent Medium Strong	3 5 7	DL 788-2, CPAN 3004 DL 803-3, GW 322 GW 89	49-51	VG
6. (+)	Plant : Flag leaf attitude	Erect Semi-erect Drooping	1 3 5	HP 1744, HD 2733 DL 784-3, HS 277 AKW 381, AKW1071	47-51	VG
7. (*)	Ear: Time of emergence (first spikelet visible on 50% of ears)	Very early Early Medium Late Very late	1 3 5 7 9	--- Sonalika, DL788-2 AKW1071, HD 2281 UP2113, HD2733 HS 277,VL 829	50-52	VG

8. (*)	Flag leaf: Waxiness of sheath	Absent Weak Medium Strong Very strong	1 3 5 7 9	--- DWR 39, K 8962 AKW 381, HD 2281 DL 788-2, HD 2327 CPAN 3004, CPAN 1676	60-65	VG
9. (*)	Flag leaf: Waxiness of blade	Absent Weak Medium Strong Very strong	1 3 5 7 9	DL 153-2 GW 173, HS207 DL 803-3, GW 322 HD 2733, HS 295 HPW 89, HS 277	60-65	VG
10. (*)	Ear: Waxiness	Absent Weak Medium Strong Very strong	1 3 5 7 9	DL 153-2, AKW 381 GW 173, HS 207 HI977, DL 788-2 HS 295, HS 1136-6-4 AKW 1071, HPW 89	60-69	VG
11. (*)	Culm: Waxiness of neck (Peduncle)	Absent Weak Medium Strong Very strong	1 3 5 7 9	K8962 HI1500, HD2402 HD 2281, K 9006 DL788-2, HS295 HD2733, HPW89	60-69	VG
12.	Flag leaf: Length	Short Medium Long	1 5 9	HD1949, PBW373 GW 322,C 306 HP89, HI 1500	70-80	MS
13.	Flag leaf: Width	Narrow Medium Broad	1 5 9	NP846,PBW373 GW 173,C306 GW89, HD2281	70-80	MS
14. (*)	Plant: Length (excluding awns/ scurs)	Very short Short Medium Long Very long	1 3 5 7 9	Lal Bahadur, HD 1949 HD 1941, K816 HD2009,DL784-3 C 306,DL 803-3 HI617, HP1493	75-92	MS

15. (*) (+)	Ear: Shape in profile	Tapering Parallel sided Club shaped Fusiform	1 2 3 4	PBW 222, PBW343 NUW533, HI 1500 HUW 234 ---	92	VS
16. (*) (+)	Ear: Density	Very lax Lax Medium Dense Very dense	1 3 5 7 9	--- Sonalika, HB 208 JAJ1 482, DL 153-2 GWR162, K 816 ---	80-92	VS-MS
17. (*)	Ear: Length (excluding awns and scurs)	Very short Short Medium Long Very long	1 3 5 7 9	VL404 NP 52 HI 617, HYB 633 K 9107 K 9644, K9006	80-92	MS
18. (*) (+)	Awns or scurs: Presence	Both absent Scurs present Awns present	1 2 3	NP 4, NP770 Ridley, NP771 PBW222, PBW343	80-92	VG
19. (*) (+)	Scurs: _____	Short tip Long tip	1 9	NP 809 Ridley	80-92	VG
20. (*)	Awns: Length	Very short Short Medium Long Very long	1 3 5 7 9	Raj Molya Rodhak VL 829,WH 542 HD 2009, CPAN 1676 HPW 89PB W 222	80-92	VG or MS
21.	Awn: Colour	White Light brown Dark brown Black	1 2 3 __	HD 2009, AKW 1071 HS 1138-6-4, DL 784-3 AKW 381, Sonalika K 68 , K 9644	80-92	VS
22. (+)	Awn: Attitude	Appressed Medium Spreading	1 2 3	HP 1493, NP 839 DL788-2, DWR 162 DL784-3, AKW1071	80-92	VS

23. (*)	Outer glume: Pubescence	Absent Medium Very strong	3 5 7	AKW381, CPAN 3004 C 306, HI 1500 DL 153-2, K 68	90-92	VS
24. (*)	Ear: Colour	White Light brown Dark brown	1 2 3	HD2009, AKW 1071 HS 1138-6-4 HD 2329, Sonalika	90-92	VG
25. (*) (+)	Lower glume: Shaller width (spikelets in mid-third of ear)	Absent or very narrow Narrow Medium Broad Very broad	1 3 5 7 9	VL 832 CPAN 1796, HD 2270 HD2281, HI 977 GW322, AKW 1071 NP 4, NP 771	80-92	VS
26. (*) (+)	Lower glume: Shaller shape (as for 25)	Sloping Round Straight Elevated Strongly elevated with 2nd point present	1 3 5 7 9	HUW 533,VL 832 HD1949, AKW 381 HD 2824, HD 2009 HP 1744, HD 2236 ---	80-92	VS
27.	Lower glume: Beak length (as for 25)	Very short Short Medium Long Very long	1 3 5 7 9	VL 738, NP 771 HD 2285, HD 2009 AKW1071, DWR 162 DL788-2, H 2270 UP 2113, K 9107	80-92	VS
28. (+)	Lower glume: Beak shape (as for 25)	Straight Moderately curved Strongly curved Geniculate	1 2 3 4	GW 322, AKW 1071 HD 2329, C 306 HUW 533, NP 839 NP 770	80-92	VS
29.	Peduncle : Length	Short Medium Long	1 5 9	HD 1941, K 816 HPW 42, DL 803-3 K 9465, HI 1500	80-92	MS

30 (+)	Spike attitude (at the time of maturity)	Straight Bent Crooked	3 5 9	Sonalika, AKW 381 RAJ 1482 Kalyansona	VG	
31. (*) (+)	Grain: Colouration with phenol	None Light Medium Dark Very dark	1 3 5 7 9	--- NI 917 C 306, HD 2281 AKW 381, DWR 162 K 9107, HD 2009	92	VG
32. (*)	Grain: Colour	White Amber Red	1 2 3	GW 89,HB 208 C 306, NP 4 HS 207, Sonora 64	92	VG
33. (*) (+)	Grain : Shape	Round Ovate Oblong Elliptical	1 2 3 4	--- CPAN 3004, GW 322 C 306, DL 153-2 PBW 222	92	VG
34. (*) (+)	Grain: Germ width	Narrow Medium Wide	3 5 7	HD 1949, DL 803-3 AKW 381, DL 803-3 WH 291, PBW 222	92	VG
35. (*) (+)	Brush hair : Length	Absent / short Medium Long	3 5 7	HD 2285, HI 617 AKW 381, PBW 373 GW 322, DL 153-2	92	VG
36. (*)	Seed : Size (weight of 1000 grains)	Small Medium Large Very large	3 5 7 9	WH 542,VL 404 GW 89, PBW 299 WH 283, LOK 1 K 68, NP 839	92	VG or MG
37. (*)	Season: Type	Winter type Alternative type Spring type	1 2 3	--- VL 616, HS 277 PBW 373, PBW 222		VG
38.	Grain : Hardness	Soft Semi-hard Hard	3 5 7	HB 208, HPW 89 HD 2329, HD 2278 NP 4, K 68		VG

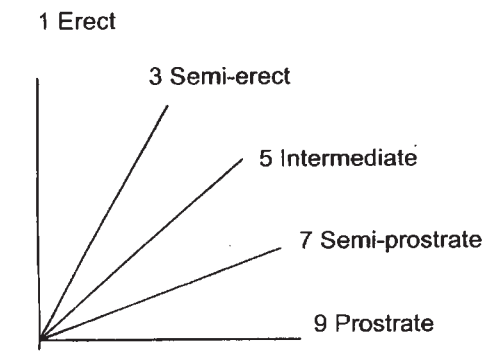
VIII. Explanations on the Table of characteristics.

Characteristic 1. Coleoptile: Method for determination of colour of anthocyanin

Number of grains per test:	20 grains for distinctness, 100 grains for homogeneity
Preparation of grains:	Set up non-dormant grains on moistened filter paper covered with a petri dish lid during germination
Place:	Laboratory
Light:	After the coleoptiles have reached a length of about 1 cm in darkness, they are placed in artificial light (daylight equivalent), at 15,000 lux continuously for 3-4 day
Temperature:	15 to 200 C
Time of recording:	Coleoptiles fully developed (about 1 week) at stage 09-11
Scale of recording:	See characteristics 1

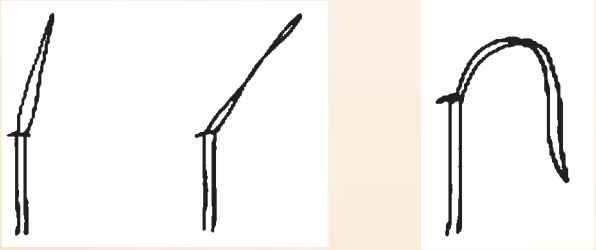
Note: At least, two of the example varieties shall be included as a control when testing for distinctness.

Characteristic 2. Plant: Growth habit



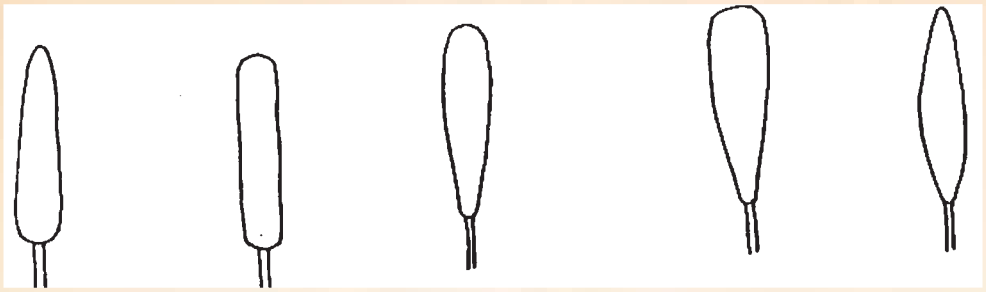
The growth habit shall be assessed visually from the attitude of the leaves and tillers.The angle formed by the outer leaves and the tillers with an imaginary vertical axis shall be used.

Characteristic 6. Plant: Flag leaf attitude



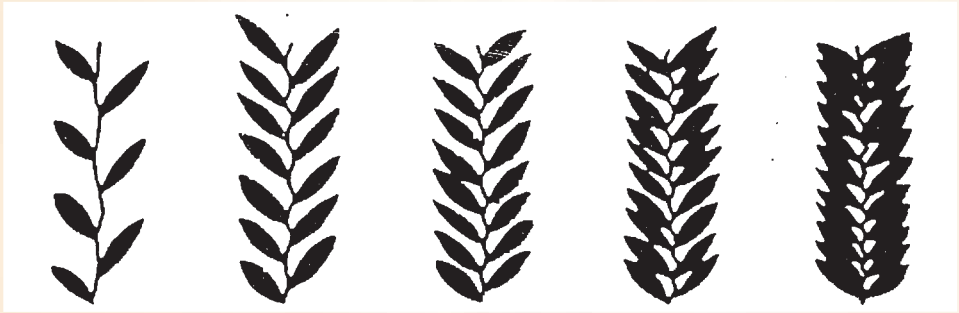
1 Erect **3 Semi-erect** **5 Drooping**

Characteristic 15. Ear: Shape in profile



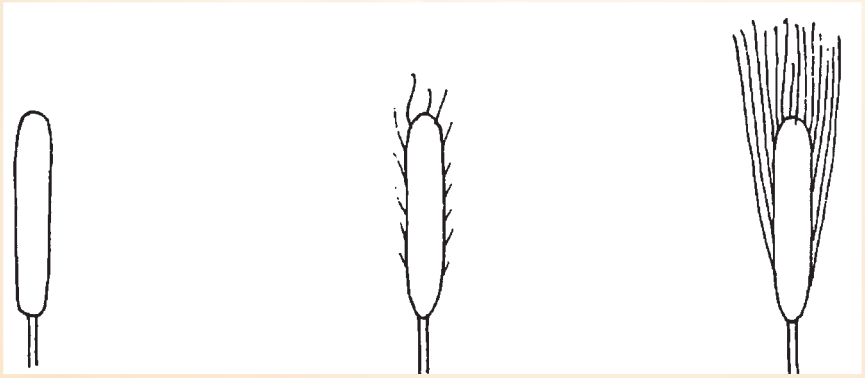
1	2	3	4	5
Tapering	Parallel sided	Semi-clavate	Clavate	Fusiform

Characteristic 16. Ear: Density



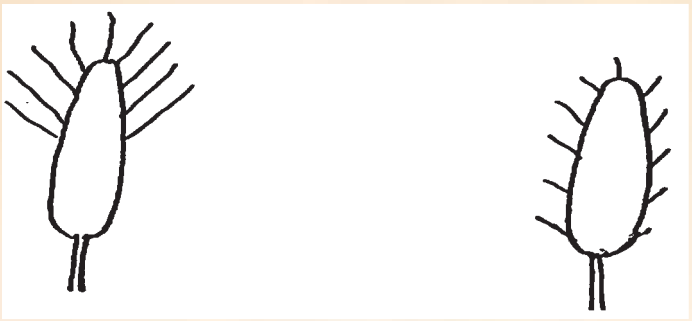
1	3	5	7	9
Very lax	Lax	Medium	Dense	Very dense

Characteristic 18. Awns or scurs: Presence



1	2	3
Both absent	Scurs present	Awns present

Characteristic 19.Scurs:



1
Scurs at tip only

9
Scurs on whole ear

Characteristic 22. Awn: Attitude

Appressed
1

Medium
2

Spreading
3

Characteristic 25. Lower glume: Shoulder width (spikelet in mid-third of ear)

1
Absent or very narrow

3
Narrow

5
Medium

7
Broad

9
Very broad

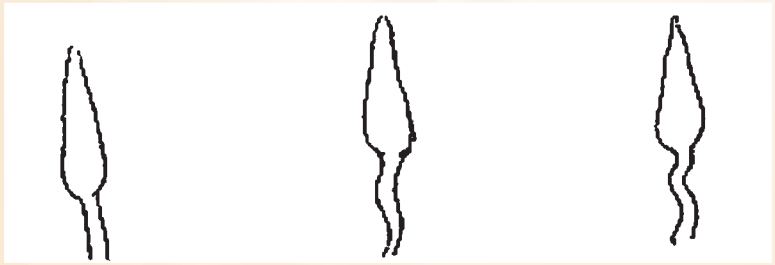
Characteristic 26. Lower glume: Shoulder shape (spikelet in mid-third of ear)

1	3	5	7	9
Sloping	Round	Straight	Elevated	Strongly elevated with 2nd point present

Characteristic 28. Lower glume: Beak shape (spikelet in mid-third of ear)

1	2	3	4
Straight	Moderately curved	Strongly curved	Geniculate

Characteristic 30. Spike attitude (at the time of flowering)



Straight	Bent	Crooked
1	2	3

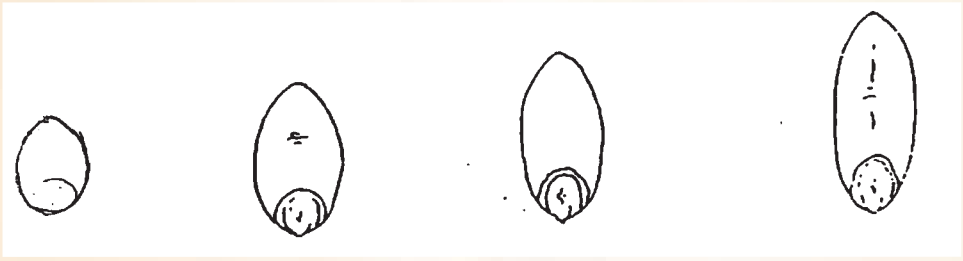
Characteristic 31. Grain: Method for colour determination of with phenol reaction

Number of grains per test: 20 grains for distinctness, 100 grains for homogeneity. The grains shall not have been treated chemically

Preparation of grains:	Soak in tap water for 16 to 20 hours, drain and remove surface water, place the grains with crease downwards, cover dish with lid
Concentration of solution:	1 per cent Phenol-solution (freshly made up)
Amount of solution:	The grains shall be about 3/4 covered
Place:	Laboratory
Light:	Daylight - out of direct sunshine
Temperature:	18 to 200C
Time of recording:	4 hours (after adding solution)
Scale of recording:	See characteristics 31 in the Table of characteristics

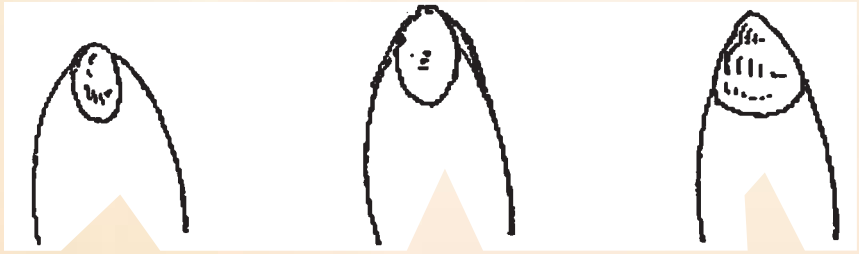
Note: At least, two of the example varieties shall be included as a control.

Characteristic 33. Grain: Shape



1	2	3	4
Round	**Ovate**	**Oblong**	**Elliptical**

Characteristic 34. Grain: Germ width



3	5	7
Narrow	**Medium**	**Wide**

Characteristic 35. Brush hair: Length

3	5	7
Absent / Short	Medium	Long

IX. Literature

IX. Working Group details

The Test Guideline developed by the National Core Committee in consultation with the Project co-ordinator (Wheat), the Nodal Officer, DUS Testing, DWR, Karnal and the Task Force (1/2005) constituted by the PPV & FR Authority

The Members of the Task Force (1/2005)

Dr. M. V. Rao (Chairman)

Dr. S. Bala Ravi

Dr. A. Seetharam

Dr. O. P. Makhija

Dr. S. P. Sharma

Dr. B. S. Dhillon

Dr. R. V. Singh

Dr. J. L. Tikkoo

Dr. (Mrs.) Malathi Laxmi Kumaran

Dr. (Mrs.) Roshini Nair

Dr. S. K. Chakrabarty

Nodal Person

Dr. (Mrs.) S. Kundu