

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

On

Maize
(Zea mays L.)



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

Government of India

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CONTENTS

	Page
I. Subject	1
II. Seed material required	1
III. Conduct of tests	1
IV. Methods and observations	2
V. Grouping of varieties	3
VI. Characteristics and symbols	3
VII. Table of characteristics	5
VIII. Explanation for the Table of characteristics	9
IX. Working Group details	13



I. Subject

These test guidelines shall apply to all varieties, hybrids and parental lines of Maize (*Zea mays* 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 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.
2. The seeds submitted shall have the following standards for germination capacity, moisture content and physical purity.
 - a. Germination capacity
 - i. Inbred lines and single cross hybrids : 80% (minimum)
 - ii. Varieties and double cross hybrids : 90% (minimum)
 - b. Moisture content : 8-10 % (maximum)
 - c. Physical purity : 98% (minimum)
3. 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. It also shall possess the highest genetic purity, uniformity, sanitary and phyto-sanitary standards.
4. The plant 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 250 plants in the plot size and planting space specified below across three replications. Separate plots for observation and 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.

4. Test plot design

Number of rows

- i. Inbred lines and single cross hybrids : 4
- ii. Varieties and other hybrids : 8

Row length : 6 m

Row to row distance : 75 cm

Plant to plant distance : 20 cm

Number of replications : 3

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

6. Additional tests 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 the testing of varieties, inbred lines and hybrids for their DUS.
2. For the assessment of Distinctiveness and Stability, observations shall be made on (excluding out-crossed plants in inbred lines and plants obviously resulting from the selfing of a parental line in single cross hybrids) at least 30 plants for inbreds/single cross hybrids and 60 plants for varieties and other hybrids.
3. For the assessment of Uniformity of inbred lines and single-cross hybrids a population standard of 1% with an acceptance probability of 95% shall be applied. In the case of a sample of 100 plants, maximum number of variants allowed would be 3 in case of inbreds and single cross hybrids and 6 in case of other varieties and hybrids. For three-way cross hybrids, double-cross hybrids and open-pollinated varieties, the variability within the variety shall not exceed the variability of comparable varieties already known.
4. All observation on ear shall be made on the upper well-developed ear.
5. For the assessment of all colour 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 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 purpose.
2. The following characteristics are proposed to be used for grouping maize varieties:
 - a) Tassel : Time of anthesis (Characteristic 4)
 - b) Ear : Time of silk emergence (50% plants) (Characteristic 11)
 - c) Ear : Anthocyanin colouration of silks (Characteristic 12)
 - d) Plant : Length (Characteristic 15.1 and 15.2)
 - e) Ear : Type of grain (Characteristic 22)

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. Notes (1 to 9) shall be given for each state of expression for different characteristics for the purpose of electronic 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.
 - (S) Characteristics may segregate in three-way cross hybrids and double cross hybrids with the effect that several states of expression occur side by side in a hybrid variety.
4. A decimal code number in the sixth column of Table of characteristics indicates the optimum stage for the observation of each characteristic during the growth and development of plant. The relevant growth stages corresponding to these decimal code numbers are described below:

Decimal Code for the Growth Stage

Stage Code	General Description
00	Dry seed
12	2 leaves unfolded
14	4 leaves unfolded
51	Inflorescence just visible
61	Beginning of anthesis
65	Anthesis halfway
71	Caryopsis watery ripe
75	Medium milk
85	Soft dough
92	Caryopsis hard (can no longer be dented by thumbnail)
93	Caryopsis loosening daytime

5. 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 plants or parts of plants

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

VS : Visual assessment by observation of individual plant 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. (+)	Leaf: Angle between blade and stem (on leaf just above upper ear)	Small (<45°)	3	HHM 2	61	VG
		Wide (>45°)	7	Vivek 5		
2. (+)	Leaf: Attitude of blade (on leaf just above upper ear)	Straight	1	HHM 2	61	VG
		Drooping	9	HKI 323		
3. (S)	Stem: Anthocyanin colouration of brace roots	Absent	1	HKI 163	65-75	VS
		Present	9	HKI 327T		
4. (*)	Tassel: Time of anthesis (on middle third of main axis, 50 % of plants)	Very early (<45 days)	1	HKI 335	65	VG
		Early (45-50 days)	3	HKI 1025		
		Medium (50-55 days)	5	HKI 323		
		Late (>55 days)	7	HKI 1126		
5. (+) (S)	Tassel: Anthocyanin colouration at base of glume (in middle third of main axis)	Absent	1	HKI 1344	65	VS
		Present	9	HKI 161		
6. (S)	Tassel: Anthocyanin colouration of glumes excluding base (in middle third of main axis)	Absent	1	HKI 209	65	VS
		Present	9	HKI 161		
7. (S)	Tassel: Anthocyanin colouration of anthers (in middle third of main axis on fresh anthers)	Absent	1	HKI 209	65	VG
		Present	9	HKI 161		
8.	Tassel: Density of spikelets (in middle third of main axis)	Sparse	3	HKI 1126	65	VG
		Dense	7	HKI 288-2		

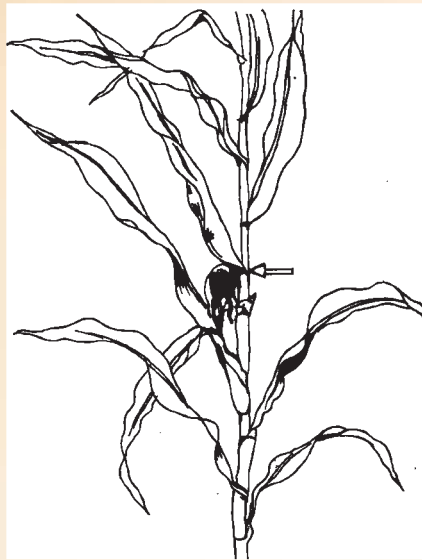
9. (* (+)	Tassel: Angle between main axis and lateral branches (in lower third of tassel)	Narrow (<45°)	3	CM 145	65	VG
		Wide (>450)	7	---		
10. (* (+)	Tassel: Attitude of lateral branches (in lower third of tassel)	Straight	1	HKI 193-1	65	VG
		Curved	5	HKI 323, 46		
		Strongly curved	9	HKI 163		
11.	Ear: Time of silk emergence (50% plants)	Very early (<48 days)	1	HKI 335	65	VG
		Early (48-53 days)	3	HKI 1025		
		Medium (53-58 days)	5	HKI 323		
		Late (>58 days)	7	HKI 1126		
12. (* (+)	Ear: Anthocyanin colouration of silks (on day of emergence)	Absent	1	HKI 1025	65	VG
		Present	9	HKI 323		
13.	Leaf: Anthocyanin colouration of sheath (below the ear)	Absent	1	HKI 163	71	VS
		Present	9	CM 300		
14.	Tassel: Length of main axis above lowest side branch	Short (<20 cm)	3	HKI 1128	71	MS
		Medium (20-30 cm)	5	HKI 327T		
		Long (> 30 cm)	7	HKI 1105		
15.1 (* (+)	Inbred lines only: Plant : Length (up to flag leaf)	Short (<120 cm)	3	HKI 1348-6-2	75	MS
		Medium (120-150 cm)	5	HKI 323		
		Long (>150cm)	7	HKI 1128		
15.2 (* (+)	Hybrids and open pollinated varieties only: Plant : Length (up to flag leaf)	Short (<150 cm)	3	HM 1	75	MS
		Medium (150-180 cm)	5	HM 4		
		Long (181-210 cm)	7	HQPM 1		
		Very long (>210 cm)	9	African Tall		

16.	Plant: Ear placement	Low	3	HKI 1011	75	MS
		Medium	5	HM 4		
		High	7	HQPM 1		
17.	Leaf: Width of blade (leaf of upper ear)	Narrow (<8 cm)	3	HKI 323	75	MS
		Medium (8-9 cm)	5	HKI 295		
		Broad (> 9cm)	7	HKI 1126		
18. (*)	Ear: Length without husk	Short (<10 cm)	3	HKI 536	92	MS
		Medium (10-15 cm)	5	HKI 163		
		Long (>15 cm)	7	HQPM 1		
19.	Ear: Diameter without husk (in middle)	Small (<4 cm)	3	HKI 323	92	MS
		Medium (4-5 cm)	5	HKI 327		
		Large (> 5 cm)	7	THQPM 1		
20. (+)	Ear: Shape	Conical	1	HKI 1344	92	VG
		Conico-cylindrical	2	HKI 295		
		Cylindrical	3	HKI 1105		
21.	Ear: Number of rows of grains	Few (£8)	3	---	92	MS
		Medium (10-12)	5	HKI 163		
		Many (³14)	7	HM 5		
22. (*)	Ear: Type of grain (in middle third of ear)	Flint	1	HKI 1105	92	VG
		Semi flint/ semi dent	2	HKI 1344		
		Dent	3	HM 5		
23. (*)	Ear: Colour of top of grain	White	1	CM 300	92	VG
		White with cap	2	HKI 1344		
		Yellow	3	HKI 1025		
		Yellow with cap	4	HKI 209		
		Orange	5	HKI 323		
		Red	6	---		
		Other (specify)	7	---		

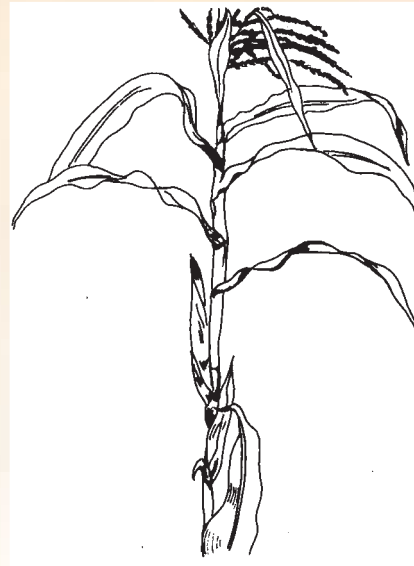
24. (*)	Ear: Anthocyanin colouration of glumes of cob	White	1	HKI 163	93	VG
		Light purple	2	HKI 295		
		Dark purple	3	HKI 161		
25. (+)	Kernel: Row arrangement (middle of ear)	Straight	1	HM2	93	VG
		Spiral	2	HM5		
		Irregular	3	HKI 1344		
26.	Kernel: Poppiness	Absent	1	HM 1		
		Present	9	Amber popcorn		
27.	Kernel: Sweetness	Absent	1	HM 1		
		Present	9	Madhuri		
28.	Kernel: Waxiness	Absent	1	HM 1		
		Present	9	---		
29.	Kernel: Opaqueness	Absent	1	HM 1		
		Present	9	CML142		
30. (+)	Kernel: Shape	Shrunken	1	Madhuri	93	VG
		Round	2	HKI 1342		
		Indented	3	HM 5		
		Toothed	4	HKI 1348-6-2		
		Pointed	5	---		
31.	Kernel: 1000 kernel weight)	Very small (<100g)	1	Madhuri	93	MG
		Small (100-200 g)	3	HKI 1025		
		Medium (200-300 g)	5	HQPM 1		
		Large (>300 g)	7	---		

VIII. Explanations for the Table of characteristics

Characteristic 1: Leaf: Angle between blade and stem (on leaf just above upper ear)



Small
3

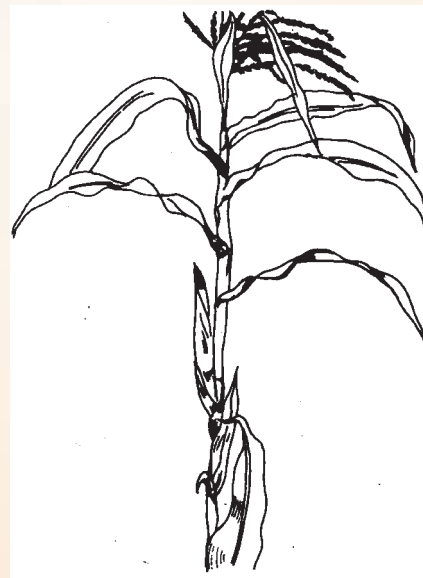


Wide
7

Characteristic 2: Leaf: Attitude of blade (on leaf just above upper ear)



Straight
1



Drooping
9

Characteristic 5 : Tassel: Anthocyanin colouration at base of glume (in middle third of main axis)

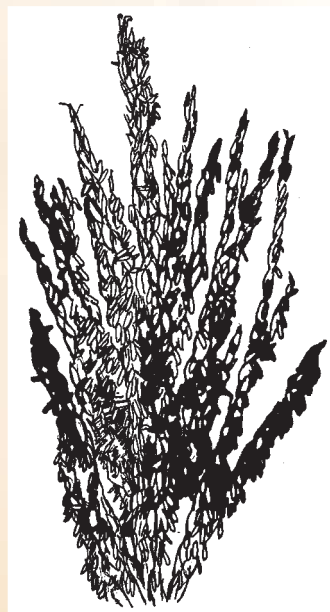


Absent
1



Present
9

Characteristic 9: Tassel: Angle between main axis and lateral branches (in lower third of tassel)



Narrow
3



Wide
7

Characteristic 10: Tassel: Attitude of lateral branches (in lower third of tassel)



Straight
1

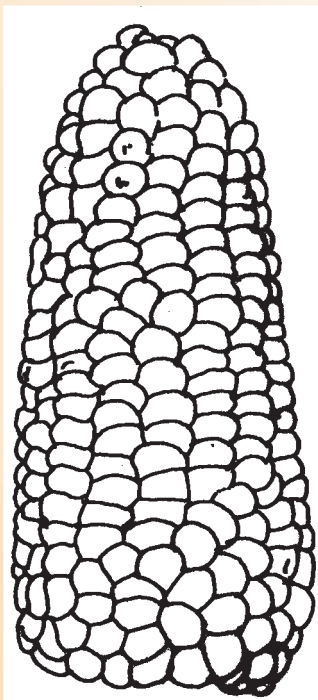


Curved
5

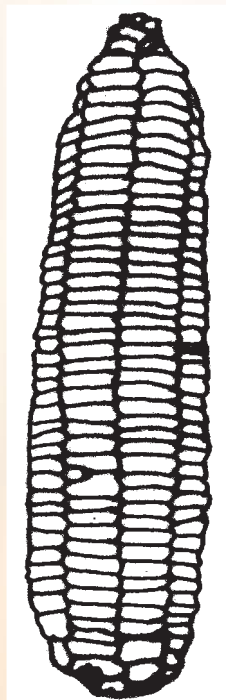


Strongly curved
9

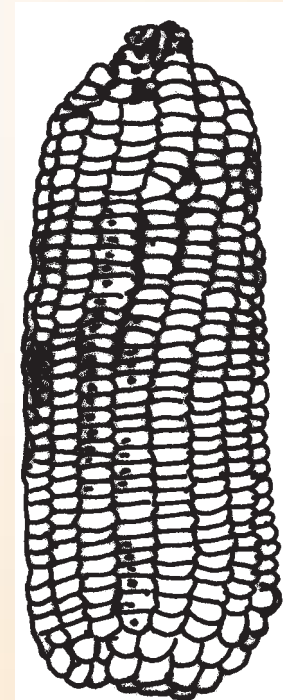
Characteristic 20. Ear: Shape



Conical
1

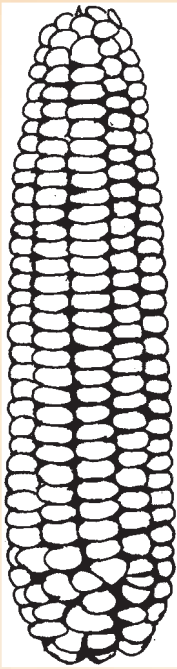


Conico-cylindrical
2

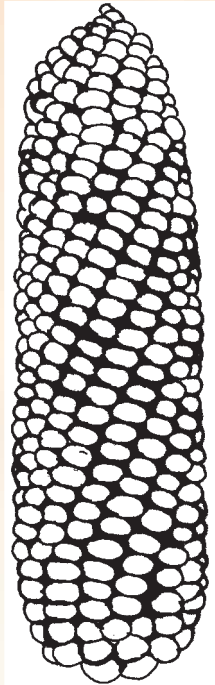


Cylindrical
3

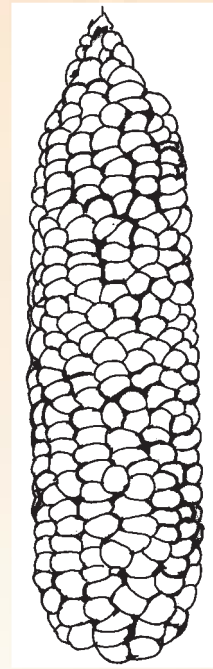
Characteristic 25. Kernel: Row arrangement (middle of ear)



Straight
1



Spiral
2

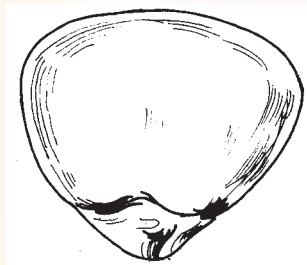


Irregular
3

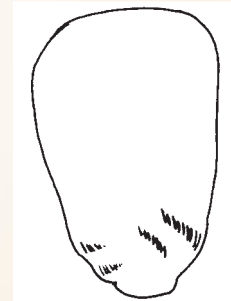
Characteristic 30. Kernel: Shape



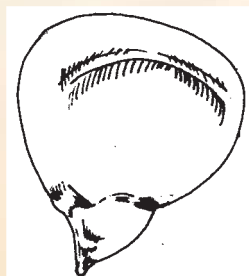
Shrunk
1



Round
2



Indented
3



Toothed
4



Pointed
5

IX. Working Group details:

The Test Guideline developed by the National Core Committee in consultation with the Project Director, Directorate of Maize Research (DMR), New Delhi, the Nodal Officer, DUS Testing, DMR, New Delhi and the Task Force (1/2005) constituted by the PPV & FR Authority

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