

Indian Jujube (Ber) (*Ziziphus mauritiana* Lamk.)

I. Subject

These test guidelines shall apply to all varieties and hybrids of *Ber* (*Ziziphus mauritiana* Lamk.).

II. Planting Material required

1. The Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) shall decide on the quantity and quality of the planting material(s) required for testing the variety and when and where it is to be delivered for registration under the Protection of Plant Varieties and Farmers' Rights (PPV & FRA) Act, 2001.
2. Applicants submitting such planting material(s) 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.
3. The minimum number of planting material to be supplied by the applicants or his/her nominee/assignee during August-September shall be 07 (seven) for each DUS Test Centre. The planting materials supplied shall be healthy, not lacking in vigour or nutrition as well as free from pests or diseases or any mechanical damage. The age of the plant(s) shall be minimum 03 months from the date of budding (propagated through shield or 'T' budding) on the *Z. rotundifolia* Lamk. rootstock commonly known as *bordi* and raised in the polythene bags (25 cm x 10 cm size) with potting mixture (2:2:1 v/v of loam soil, compost and fine sand).
4. The planting material(s) shall not have undergone any treatment (chemical/bio-physical or others) which would affect the expression of the characteristics of the variety, unless the Competent Authority allow or request for such treatment. If it has been treated, full details of the treatment must be given.

III. Conduct of tests

1. The minimum duration of the tests shall normally be at least two independent similar fruiting seasons in different years. Tests shall be conducted at least at two places. If any essential characteristic 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 by the applicant, for which additional quantity of planting material shall be required.
2. The tests should be carried out under favourable conditions ensuring normal growth for the expression of the relevant characteristics of the variety and for the conduct of the tests. In particular, it is essential that the plants produce a satisfactory crop of fruit in each of the two growing cycles.

3. Test plot design: The design of the tests should be such that plants or parts of plants may be removed for measurement or observation without prejudice to the observations which must be made up to the end of the growing cycle. The additional test protocol for special purpose may be established by PPV & FRA. As a minimum, each test shall include five plants per location, planted at DUS test centre, with a spacing of 4m x 4m.
4. Plant and fruit characters will be assessed to include two similar harvest seasons.
5. On-site DUS testing:
 - The applicant or his/her nominee on his/her behalf shall submit a request to the Authority for conducting a reliable trial according to Test Guidelines and the instructions from Authority before on-site examination of the candidate variety.
 - The applicant or his/her nominee shall submit a request to the Authority for on-site examination prior to start of growing cycle as mentioned in Test Guidelines for site examination of the candidate variety.
 - On-site testing may be conducted at the places specified by the applicant. The age of the trees at on-site shall be minimum 3 years.
 - As a minimum, 05 trees planted in uniform spacing, should be available for inspection and examination for 'on site' DUS testing. The trees must be healthy and free from pest & disease and raised under standard management practices. For farmer's variety or landraces, the authority may notify suitable guidelines on the number of plant(s) and season(s), if any.
 - On-site examination shall be arranged during the fruiting season, when distinguishing characteristics of candidate variety can most easily be seen. The characteristics of the candidate variety can be examined and compared with those of the comparative varieties as per the Test guidelines.
 - The Expert Committee constituted by the PPV & FRA in consultation with the DUS Centre shall be authorized to inspect on-site testing and recording of the appropriate characters.
 - Applicant shall supply the Expert Committee with summary of distinct characteristics supported by photographs. The Expert Committee shall take notes and

observations on distinctness and shall confirm preliminary data and/or summary of distinctness from applicant.

- The Expert Committee shall submit examination report to the Authority.

IV. Methods and observations

The characteristics described in the Table of characteristics (see section VII) shall be used for the testing varieties and hybrid for their DUS.

1. For the assessment of Distinctiveness and Stability observation shall be made on 5 plants or parts taken from each of 5 plants. In the case of parts of plants, the number to be taken from each of the plants should be 2.
2. Fully mature leaves, not showing the sign of active growth, in the middle of tertiary branches should be selected for the observations on the leaf.
3. Observations on thorn should be recorded in the middle of tertiary branches.
4. Observations on the mature fruit should be recorded when fruit is ready for harvesting.
5. For assessment of all colour characteristics, the 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.

The following characteristics are to be used for grouping *ber* varieties:

- a. Growth habit (Characteristic 1)
- b. Leaf: Shape (Characteristic 5)
- c. Fruit maturity group (Characteristic 14)
- d. Mature fruit: Shape (Characteristic 18)
- e. Mature fruit: Colour (Characteristic 22)
- f. Pulp texture (Characteristic 24)
- g. Stone shape (Characteristic 28)

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.

4. Type of assessment of characteristics indicated in column seven of Table of Characteristics are as follow:

MG: Measurement by single observation of a group of plants or part of plants.

MS: Measurement of a number of individual plants or part 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 part of plants.

5. A 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 code numbers are described below:

a) Observation on growth habit, shoot surface, thorn and leaf characters should be recorded three months after pruning, when canopy attains its characteristic shape. Fully mature leaves, not showing the sign of active growth, in the middle of tertiary branches should be selected for the observations on the leaf.

b) Observation on immature fruit should be recorded when fruit has not attained its full size and is predominantly green and quite hard in texture.

c) Observations on the mature fruit and stone should be recorded when fruit is ready for harvesting.

VII. Table of characteristics

S. No.	Characteristics	States	Notes	Example variety	Stage of observation	Type of assessment
1	2	3	4	5	6	7
1. (* (+)	Growth Habit	Erect Semi-erect Spreading	1 3 5	Tikadi Chhuhara Gola	a	VG
2. (+)	Shoot surface	Smooth Tomentose	3 5	Tikadi Gola	a	VG
3. (+)	Leaf: Apex	Acute Obtuse	3 5	Jogia Umran	a	VG
4. (+)	Leaf: Base	Acute Cordate Round Oblique	1 2 3 4	Dharki No. 1 Mehrun Mundia Kaithali	a	VG
5. (* (+)	Leaf: Shape	Ovate Oval Cordate Obovate	1 3 5 7	Safeda Rohtak Gola Kaithali Kismis	a	VG
6. (+)	Leaf: Curving	Absent Present	1 9	Tikadi Umran	a	VG
7. (+)	Leaf: Pubescence on lower surface	Smooth Sparsely tomentose Densely tomentose	1 3 5	Tikadi Banarasi Karaka Seb	a	VG
8. (+)	Leaf blade: Length (cm)	Short (<7) Medium (7-9) Long (>9)	3 5 7	Illaichi Banarasi Pewandi Mundia	a	MS
9. (+)	Leaf blade: Width (cm)	Narrow (<6) Medium (6-7) Broad (>7)	3 5 7	Tikadi ZG-3 Mundia	a	MS
10.	Branch: Thorniness	Less Medium High	3 5 7	Illaichi Chhuhara Tikadi	a	VG
11. (+)	Thorn: Shape	All Curved Alternate Curved	3 5	Mehrun Gola	a	VG
12.	Thorns	Non-	1	Kaithali	a	VG

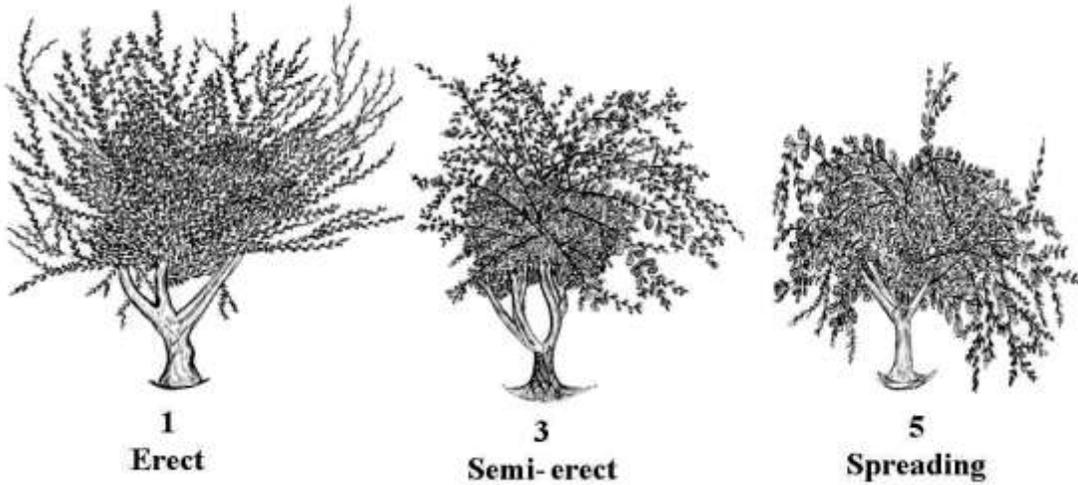
	persistence: Cauducous	Persistent(Absent) Persistent(Present)	9	Banarasi Pewandi		
13.	Immature fruit: Anthocyanin blush	Absent Present	1 9	Chhuhara Kathaphal	b	VG
14. (* (+)	Fruit maturity group	Early Mid Late	3 5 7	Gola Seb Mehrur	c	VG
15. (+)	Bearing habit: Bunching	Absent Present	1 9	Umran Illaichi	c	VG
16. (+)	Fruit cracking	Absent Present	1 9	Gularvasi Gola	c	VG
17. (+)	Fruit shape: Apex	Flat Round Pointed	1 3 5	Illaichi Umran Banarasi Pewandi	c	VG
18. (* (+)	Mature Fruit: Shape	Oblong Oval Ovate Oblate Round Falcate	1 2 3 4 5 6	Mehrur Chhuhara ZG-3 Illaichi Gola Narma	c	VG
19. (+)	Mature fruit: Length (mm)	Short (<20) Medium (20-30) Long (>30)	3 5 7	Dharki No. 1 Mehrur Chhuhara	c	MS
20. (+)	Mature fruit: Width (mm)	Narrow (<15) Medium (15-25) Broad (>25)	3 5 7	Dharki No. 1 Illaichi Gola	c	MS
21.	Mature fruit: Weight (g)	Very Low (<5) Low (5-10) Medium (11-20) High (21-30) Very High (>30)	1 3 5 7 9	Mehrur Illaichi Seb Umran Sanaur-5	c	MS
22. (* (+)	Mature fruit: Colour	Yellow (12A) Greenish Yellow (154A) Chocolate Brown (46B)	3 5 7	Gola ZG-3 Tikadi	c	VG
23. (+)	Fruit surface	Plain Ridged & Wart	3 5	Gola Umran	c	VG

24. (* (+)	Pulp texture	Soft Medium Hard	3 5 7	Chhuhara ZG-3 Umran	c	VG
25. (+)	Pulp cavity	Absent Present	1 9	Sanaur-5 Chhuhara	c	VG
26. (+)	Pulp cavity: Stylar end	Absent Present	1 9	Gola Chhuhara	c	VG
27. (+)	Pulp cavity: Stem end	Absent Present	1 9	Tikadi Gularvasi	c	VG
28. (+)	Stone shape: Apex	Obtuse Acute	3 5	Seb Kaithli	c	VG
29. (* (+)	Stone shape	Oblong Oval Spindle Club Falcate	1 2 3 4 5	Seb Gola Sanaur-5 Chhuhara Chhuhara Bawal	c	VG
30. (+)	Stone: Length (mm)	Short (<15) Medium (15-25) Long (>25)	3 5 7	Illaichi Gola ZG-3	c	MS
31. (+)	Stone: Width (mm)	Narrow (<7) Medium (7-9) Broad (>9)	3 5 7	Illaichi Umran Seb	c	MS
32.	Stone: Weight (g)	Very Low (<1.0) Low (1-2) Medium (2.1-3) High (3.1-4) Very High (>4)	1 3 5 7 9	Illaichi Gola ZG-3 Safeda Selection Sanaur-5	c	MS
33. (+)	Pulp: stone ratio	Very Low (<5) Low (5-10) Medium (11-15) High (16-20) Very High (>20)	1 3 5 7 9	Mehrun ZG-3 Gola Chhuhara Kala Gola	c	MS
34. (+)	Pulp TSS (°Brix)	Less (<10) Medium (10-20) High (>20)	3 5 7	Dharki No.1 Gola Reshmi	c	MS
35. (+)	Pulp acidity (%)	Less (<0.3) Medium (0.3-0.5) High (>0.5)	3 5 7	Chhuhara Gola Chhuhara Bawal	c	MS

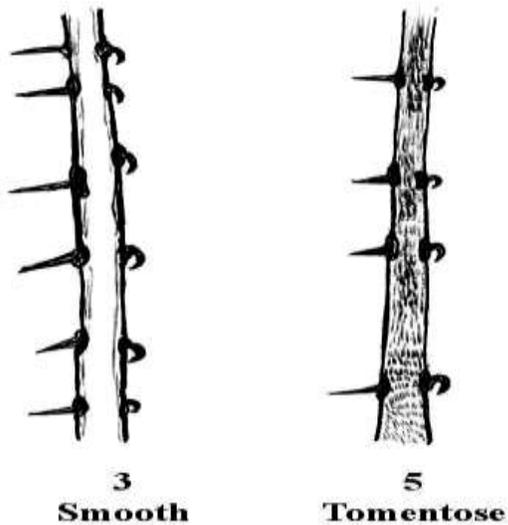
36. (+)	Ascorbic acid content of pulp (mg/100g FW)	Very Less (<50)	1	Kala Gola	c	MS
		Less (50-100)	3	Gola		
		Medium (101-150)	5	Banarasi Pewandi		
		High (150-200)	7	Sanaur-5		
		Very High (>200)	9	Lakhan		

VIII. Explanation for the Table of Characteristics

Characteristic 1: Growth habit



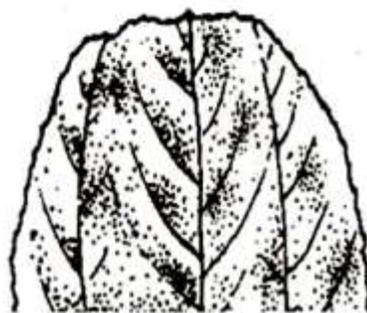
Characteristic 2: Shoot surface



Characteristic 3: Leaf: Apex

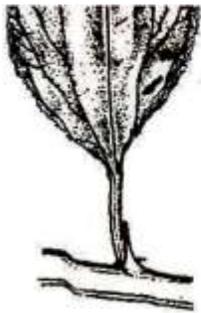


3
Acute

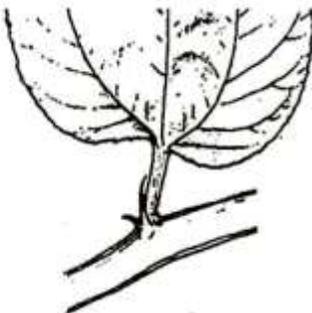


5
Obtuse

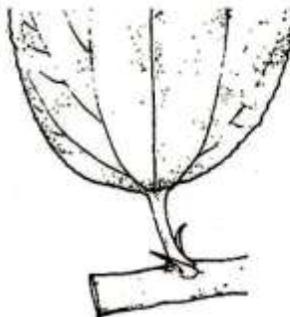
Characteristic 4: Leaf: Base



1
Acute



2
Cordate

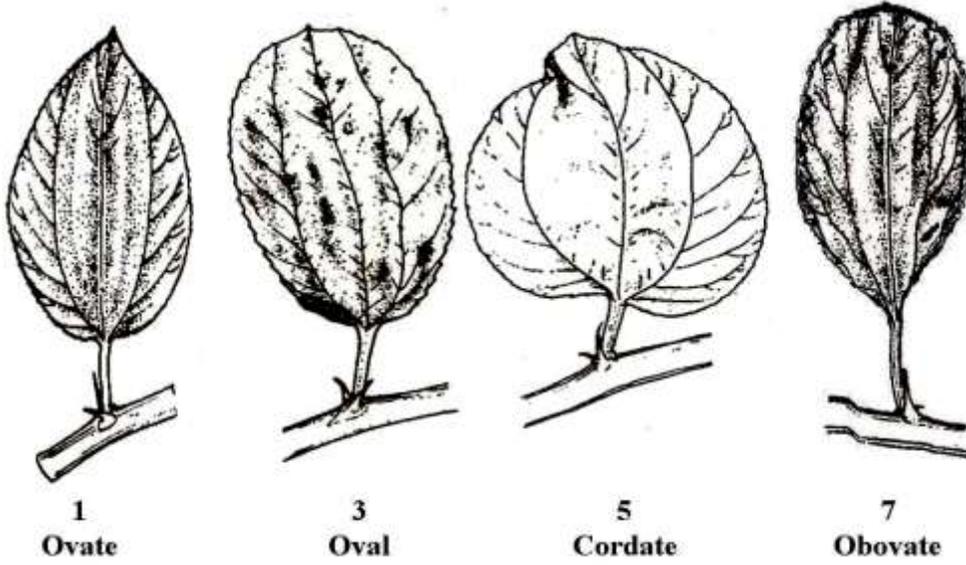


3
Round

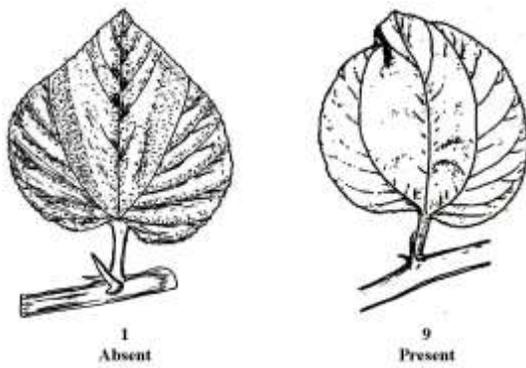


4
Oblique

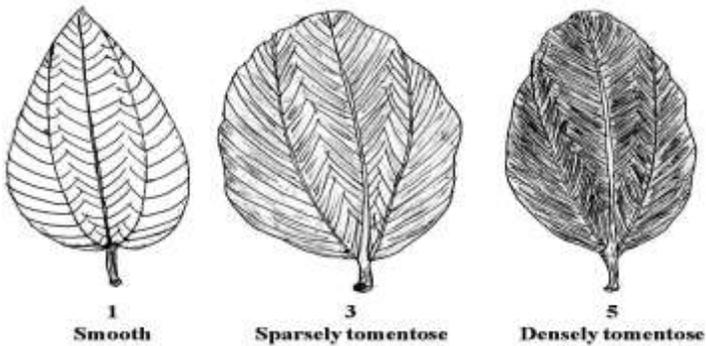
Characteristic 5: Leaf: Shape



Characteristic 6: Leaf: Curving

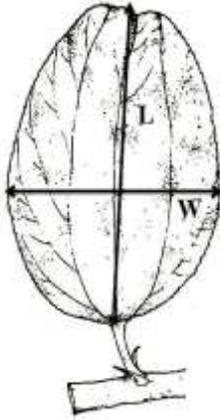


Characteristic 7: Leaf: Pubescence on lower surface



Characteristic 8: Leaf blade: Length (L)

Characteristic 9: Leaf blade: Width (W)



Characteristic 11: Thorn: Shape

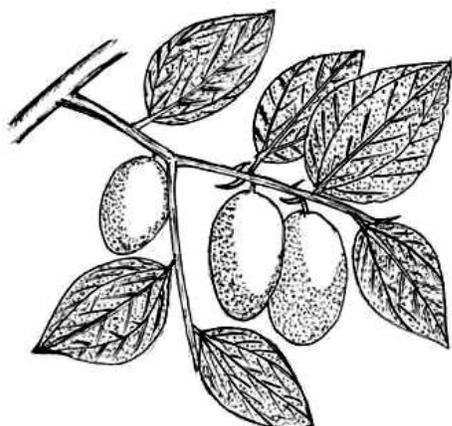


3
All Curved

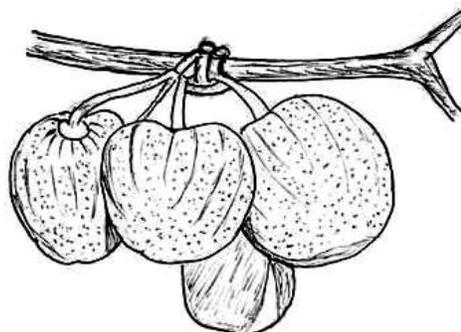


5
Alternate Curved

Characteristics 15: Bearing habit: Bunching



1
Absent



9
Present

Characteristic 16: Fruit cracking

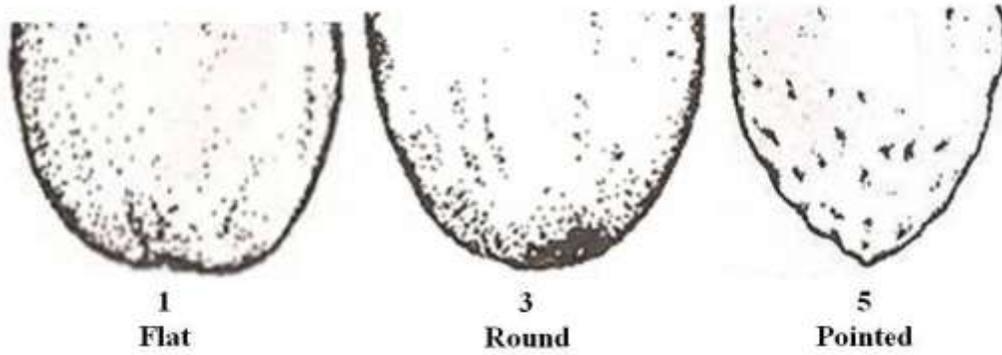


1
Absent

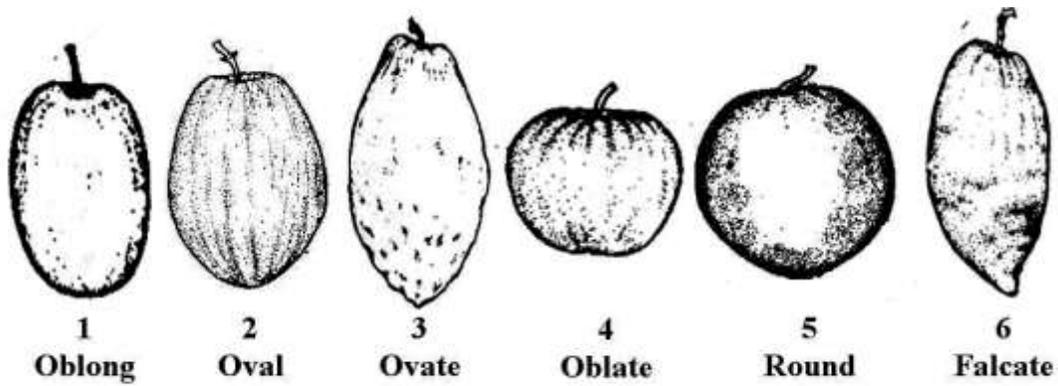


9
Present

Characteristic 17: Fruit shape: Apex

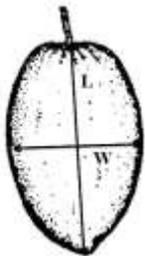


Characteristic 18: Mature Fruit: Shape

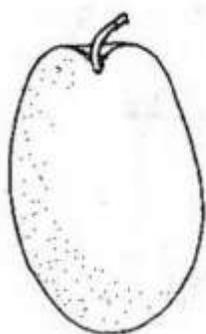


Characteristic 19: Mature Fruit: Length

Characteristic 20: Mature Fruit: Width



Characteristic 23: Fruit Surface



3
Plain

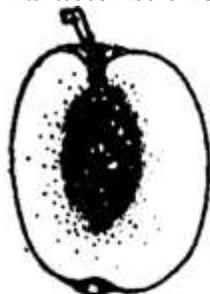


5
Ridged and
wart

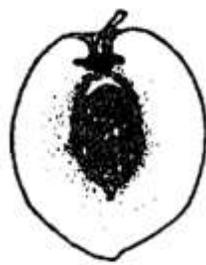
Characteristic 24: Pulp texture

Pulp texture shall be determined by eating the fresh fruits, which shall involve senses of touch and pressure. With the sensory method, the evaluation shall include various steps inside the mouth, from the first bite through mastication, swallowing and residual feel in the mouth and throat.

Characteristic 25: Pulp cavity

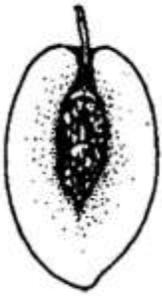


1
Absent



9
Present

Characteristic 26: Pulp cavity: Stylar end

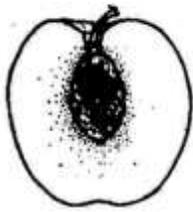


1
Absent

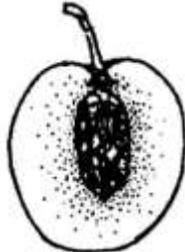


9
Present

Characteristic 27: Pulp cavity: Stem end



1
Absent



9
Present

Characteristic 28: Stone Shape: Apex

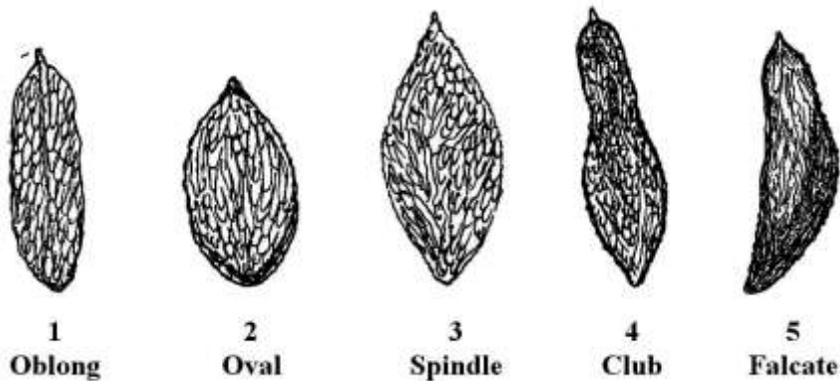


3
Obtuse



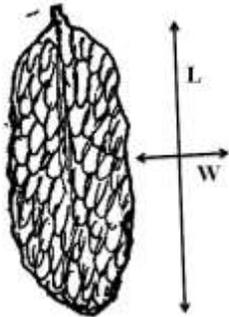
5
Acute

Characteristic 29: Stone Shape



Characteristic 30: Stone: Length

Characteristic 31: Stone: Width



Characteristic 33: Pulp: Stone ratio

The stone shall be removed from the fruit and pulp & stone shall be weighed separately. The Pulp: Stone ratio shall be determined by following formulae;

$$\text{Pulp: Stone ratio} = \frac{\text{Average pulp weight (g)}}{\text{Average stone weight (g)}}$$

Characteristic 34: Pulp TSS (⁰Brix)

The fruits of the variety under test shall be harvested as per uniformity in size, shape and colour at maturity stage. For determination of total soluble solid (TSS), twenty gram fruit pulps (20 g) shall be blended for 3 min. Followed by wrapping in cheesecloth, squeezing by hand and

then expressing juice used for measurement of TSS in ⁰Brix using hand-held/ digital refractometer (Krishna and Parashar, 2013).

Characteristic 35: Pulp acidity (%)

The pulp acidity contents of the samples shall be determined by visual titration method as suggested by Ranganna (1986) with slight modification. For estimation of total acidity in samples, twenty gram (20 g) fruit pulp shall be blended and mixed thoroughly. Later, it shall be filtered and transferred to volumetric flask to make up the volume to 100 ml. Ten-milliliter aliquot of the sample prepared as above shall be titrated with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.1. The content shall be expressed as percentage of citric acid.

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Volume made up} \times \text{Equivalent weight of acid (i.e. 64)} \times 100}{\text{Volume of sample taken for estimation} \times \text{Weight or volume of sample taken} \times 1000}$$

Characteristic 36: Ascorbic acid content of pulp (mg/100g FW)

The ascorbic acid contents of the samples shall be determined by visual titration method of reduction of 2, 6-dichlorophenol–indophenol dye as per the method suggested by Ranganna (1986). Results shall be expressed as mg/100 g FW.

Reagents

(a) Ascorbic acid standard: Weigh accurately 100 mg of L-ascorbic acid and make up to 100 ml with 3% HPO₃. Dilute 10 ml to 100 ml with 3% HPO₃ (1 ml = 0.1 mg of ascorbic acid).

(b) Dye solution: Dissolve 50 mg of the sodium salt of 2, 6-dichlorophenol–indophenol (C₁₂H₆C₁₂NNaO₂.2H₂O) in approximately 150 ml of hot glass distilled water containing 42 mg of sodium bicarbonate (NaHCO₃). Cool and dilute with distilled water to 200 ml.

For standardization of dye, five ml each of standard ascorbic acid solution and HPO₃ shall be taken together and shall be titrated with the dye solution to a pink colour, which should persist for 15 sec. The dye factor (mg of ascorbic acid/ ml of dye) shall be calculated using following formulae;

$$\text{Dye factor} = \frac{0.5}{\text{Titre}}$$

For estimation of ascorbic acid in fruit sample, five grams of pulp shall be taken and blended with 3% meta-phosphoric acid (HPO₃). The final volume shall be made upto 100 ml with HPO₃ followed by centrifugation or filtration. Two ml aliquat of the HPO₃ extract of the pulp shall be taken titrated with standard dye to a pink end-point, which should persist for at least

15 sec. Calculation of ascorbic acid content of the sample shall be done from the following formulae;

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Aliquot of extract taken for estimation} \times \text{Weight of sample taken for estimation}}$$

XI. DUS Test Centres

Nodal Centre

Central Institute for Arid Horticulture,
Beechwal, Bikaner-334 006, Rajasthan

Other Centre

MPKV, Rahuri (subject to confirmation of
PPV&FRA)