GUIDELINES

FOR THE CONDUCT OF TEST FOR

DISTINCTIVENESS, UNIFORMITY AND STABILITY OF

BAEL

(*Aegle marmelos* Correa)

Protection of Plant Varieties and Farmer's Right Authority
(PPV&FRA)
Government of India

CONTENTS

Bael (*Agle marmelos* Correa)

I. Subject

These test guidelines shall apply to all varieties and hybrids of bael *(Aegle marmelos* Correa*).*

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. 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. The minimum number of planting material to be supplied by the applicants or his/her nominee/assignee during July-August shall be 05 (five) for each DUS Test Centre.
2. 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 06 months from the date of budding (propagated through patch budding) raised in the polythene bags (25 cm x 10 cm size) with potting mixture (2:2:1 ratio of loam soil, compost and fine sand).The root stocks should be raised from the seeds of single fruit or developed through stooling.
3. 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 mentioned explicitly.

III. Conduct of tests

1. The minimum duration of the DUS tests shall normally be at least two independent similar fruiting seasons in different years.

2. The 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. In case any unforeseen situation, the data from third fruiting season may also be considered for testing.

3. 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.

4. 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 the minimum, each test shall include five plants per location, planted at DUS test centre, with a spacing of 8m x 8m.

5. Each test shall include five plants per location, planted at DUS test centre with a spacing of 8m x 8m.
6. The additional test protocol for special purpose may be established by PPV & FRA.

7. 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 5 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 and uniform 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 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 validation of recorded DUS 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 to the authority of PPV &FRA, New Delhi.

IV. Methods and observations

1. The characteristics described in the Table of characteristics (see section7) shall be used for the testing varieties and hybrid for their DUS.
2. For the assessment of Distinctiveness and Stability observations shall be made on 05 plants or parts taken from each of 05 plants. In the case of parts of plants, the number to be taken from each of the plants should be 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 the mature fruit should be recorded when fruit is ready for harvesting.
4. For assessment of all colour characteristics, the Royal Horticultural Society (RHS) colour chart shall be used.

V. Grouping of varieties

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 *bael* varieties:

a. Growth habit (Characteristic 1)

b. Leaf characters (Characteristic 5-11)

c. Flower characters (Characteristic 17, 18 &19)

d. Fruit Shape (Characteristic 23)

e. Fruit characters (Characteristic 30, 32, 33&35)

VI. Characteristics and symbols

1. To assess Distinctiveness, Uniformity and Stability, the characteristics and their states as given in the Table of characteristics (Section 7) shall be used.
2. Notes (l 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 7. 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 by a single observation 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 and leaf characters should be recorded after four to five (September-October) months of leaf shedding, when canopy attains its characteristic shape.

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

c) Observations on the mature fruit should be recorded when 40 per cent fruit is ready for harvesting.

VII. Table of characteristics

Sr. No.	Characteristics	State	Note	Example variety	Stage of observation	Type of assessment
1	2	3	4	5	6	7
1 (*) (+)	Growth habit	Drooping	**1**	Pant Aparna, Pant Shivani	**a**	**VG**
		Spreading	**3**	Pant Urvashi, NB-9, NB-16,		
		Semi-spreading	**5**	CISHB-2, Pant Sujata, NB-5, NB-7, Goma Yashi,		
		Upright	**7**	CISHB-1, NB-17		
2. (*)	Foliage	Sparse	**1**	CISHB-2, NB-16, NB-17, Pant Shivani, Pant Aparna	**a**	**VG**
		Dense	**9**	CISHB-1, Pant Sujata, Pant Urvashi, NB-5 NB-9, Goma Yashi		

3. (*) (+)	Phyllotaxy		Tristichous	**1**	CISHB-2, Pant Sujata, NB-5, NB-9, NB-16, Goma Yashi	**a**	**VG**
			Pentasticshous	**9**	CISHB-1, Pant Aparna, Pant Urvashi, NB-7, NB-17		
4. *	Inter nodal distance (cm)		Low (<3 cm)	**3**	NB-5,	**a**	**MG**
			Medium 3-3.50 cm	**5**	Pant Sujata, Pant Urvashi, NB-17, GomaYashi, NB-16		
			High >3.50 cm	**7**	NB-9, NB-7, CISHB-1, CISHB-2,Pant Aparna, Pant Shivani,		
5 *	Leaf size		Small	**3**	Pant Urvashi, NB-9, NB-17, NB-5	**a**	**MS**
			Medium	**5**	Pant Sujata, Goma Yashi, Pant Aparna		
			Large	**7**	NB-7, Pant Shivani		
6 (*) (+)	Leaf shape	Central leaflet	Broadly lanceolate to ovate	**1**	CISHB-1	**a**	**VG**
			Ovate	**3**	Pant Aparna, Pant Urvashi, Pant Shivani, NB-17, CISHB-2		
			Ovate to elliptic	**5**	NB-5		
		Lateral Leaflet	Ovate	**1**	CISHB-1,CISHB-2,Pant Aparna, Pant Urvashi, Pant Shivani, NB-9,NB-		

					17		
			Elliptical	**3**	NB-5,		
			Lanceolate	**5**	Goma Yashi		
7. (*) (+)	Leaf apex		Acuminate	**3**	CISHB-1, CISHB-2,	**a**	**VG**
			Acute	**7**	Pant Aparna, Pant Sujata, Pant Shivani, NB-16,		
			Aristate	**9**	Goma Yashi		
8 * (+)	Leaf base		Narrowly Cuneate	**1**	CISHB-1,Pant Sujata, NB-5, NB-16 Pant Aparna, Goma Yashi	**a**	**VG**
			Round	**5**	CISHB-2, Pant Urvashi, Pant Shivani, NB-9, CISHB-1,		
			Attenuate	**7**	NB-7		
9. *	Leaf Surface		Smooth	**1**	NB-7, Pant Aparna, Pant Sujata, Pant Urvashi, NB-7, NB-9, NB-16	**a**	**VG**
			Rough	**9**	CISHB-1,CISHB-2,Pant Shivani, NB-5, NB-17,		
10. *	Leaf length (cm)		Small < 20 cm	**1**	CISHB-1, Pant Urvashi, NB-5, NB-9, Goma Yashi	**a**	**MS**
			Large>20-cm	**9**	CISHB-2, Pant Aparna, Pant Sujata,NB-17, NB-16, Pant Shivani, NB-7		

11. *	Leaf width (cm)	small <18 cm	**1**	NB-16, Goma Yashi, NB-17,NB-5,Pant Urvashi,	**a**	**MS**
		Large>18 cm	**9**	CISHB-2,CISHB-1,NB-9,Pant Aparna, NB-7		
12. (*)	Trunk colour	Yellowish grey	**1**	CISHB-1, Pant Shivani,NB-5,NB-17	**a**	**VG**
		Yellow	**3**	CISHB-2,Pant Aparna,		
		Greyish yellow	**5**	Pant Sujata, Pant Urvashi,		
		Grey	**7**	NB-7, NB-9, NB-16		
13. (*)	Bark splitting pattern	Rectangular	**3**	NB-5,NB-17	**a**	**VG**
		Cylindric	**5**	CISHB-1,Pant Shivani, CISHB-2,Pant Urvashi,		
		Irregular	**7**	Pant Aparna,NB-16,NB-9, Pant Sujata, NB-7,		
14 (*).	Leaf colour	Light green	**3**	Pant Shivani, Pant Urvashi,	**a**	**VG**
		Green	**5**	CISHB-1,NB-16,CISHB-2,NB-17, Goma Yashi		
		Dark green	**7**	NB-5, Pant Aparna,NB-16,		
15. (*) (+)	Leaf margin	crenulate	**3**	CISHB-1,Pant Urvashi, Pant Sujata	**a**	**VG**
		crenate	**7**	Pant Shivani, NB-		

				5, NB-9, CISHB-2, Pant Aparna,		
16. (*) (+)	Thorniness at basal portion of primary branches	thornless	**1**	Pant Shivani, Goma Yashi,	**a**	**VS**
		thorny	**9**	CISHB-1,NB-5, Pant Aparna, NB-16, NB-9, Pant Sujata, NB-17, NB-7, CISHB-2,		
17 (+) .	Inflorescence type	Axillary biparous cyme	**1**	Pant Aparna, Pant Urvashi, CISHB-1,CISHB-2,NB-7,	**a**	**VG**
		Axillary multiparous cyme	**3**	Pant Sujata,Nb-17,Goma Yashi,		
		Terminally biparous cyme	**5**	Pant Shivani		
		Terminally multiparous cyme	**7**	NB-16, NB-5		
		Axillary Uniparous	**9**	NB-9		
18 (*)	Inflorescence length	Small<8cm	**1**	Pant Shivani CISHB-1, Pant Urvashi, NB-9, Goma Yashi, NB-17	**a**	**MS**
		Light green	**3**	NB-5,NB-17		
19 .(*)	Flower size	Small <12	**1**	NB-9,Goma Yashi	**a**	**MS**
		Medium12-15 cm	**5**	CISHB-1,NB-5, NB-16,Pant Shivani, Pant Urvashi		
		Large	**9**	CISHB-2,NB-		

		>15 cm		7,NB-17,NB-16, Pant Sujata		
20. (*)	Maturity of fruit	Early (After 280 days of fruit setting))	**3**	CISHB-1,Pant Shivani, Goma Yashi	**c**	**VG**
		Mid (after 310 days of fruit setting)	**5**	Pant Sujata, Pant Urvashi, NB-9, Pant Aparna		
		Late (after 340days of fruit setting)	**7**	CISHB-2, NB-5, NB-7		
21. (*)	Immature fruit colour	Light green	**3**	CISHB-1, NB-7 Pant Shivani, Goma Yashi	**b**	**VG**
		Green	**5**	CISHB-2, NB-5, NB-16, Pant Urvashi, Pant Sujata		
		Dark green	**7**	NB-9, Pant Aparna		
22. (*) (+)	Mature fruit colour	Green	**3**	NB-16, Pant Sujata, NB-9, CISHB-2	**C**	**VG**
		Greenish pale yellow	**5**	NB-5, Goma Yashi		
		Yellowish Green	**7**	CISHB-1, Pant Aparna, Pant Shivani, NB-17, Pant Urvashi, NB-7		
23. (*) (+)	Fruit Shape	Globose	**1**	Goma Yashi, Pant Shivani, Pant Urvashi	**C**	**VS**
		Ovate	**3**	CISHB-1, NB-9,		
		Elliptical	**5**	CISHB-2, NB-7, Pant Urvashi		
		Round	**9**	Pant Aparna, NB-16, Pant Sujata		

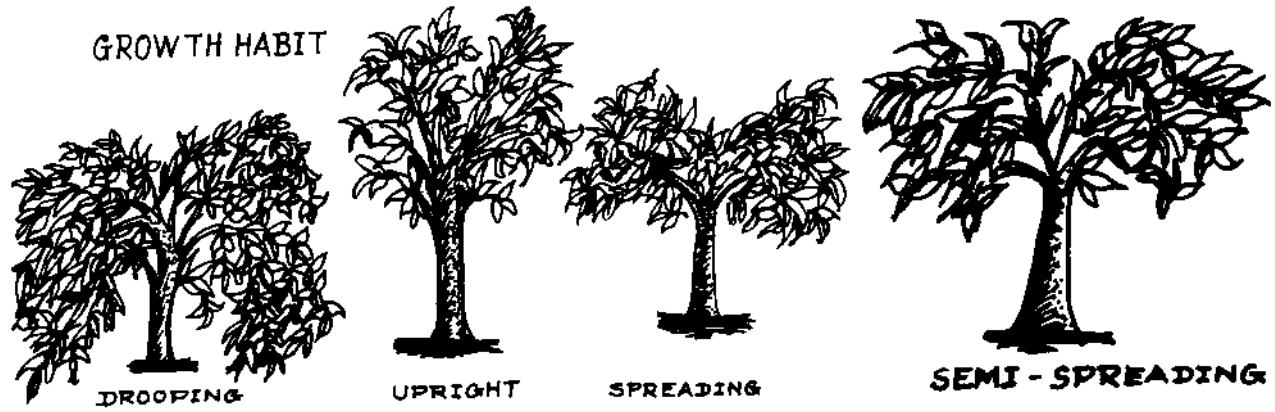
28. (*)	Fruit Surface	Smooth	**1**	NB-5,NB-7, Pant Shivani, Goma Yashi CISHB-2,CISHB-1, Pant Urvashi, Pant Aparna	**C**	**VG**
		Rough	**9**	NB-9,NB-16, Pant Sujata		
29. (*)	Pulp colour	Pale yellow	**1**	NB-5, NB-7, NB-16, CISHB-2	**C**	**VG**
		Yellow	**2**	Pant Shivani, Pant Sujata, Pant Aparna, Goma Yashi, NB-17		
		Dark yellow	**3**	CISHB-1, NB-9, Pant Urvashi,		
30. (*)	Shell thickness	Thin < 2mm	**1**	GomaYashi, NB-5, CISHB-1,	**C**	**VG**
		Thick >2mm	**9**	N B-7, NB-9, Pant Urvashi, NB-16, CISHB-2, Pant Aparna, Pant Sujata, NB-17, Pant Shivani,		
31. (*)	Mucilage	Low	**3**	Goma Yashi, CISHB-1,NB-5,NB-17	**C**	**VS**
		High	**5**	NB-9, NB-16, Pant Sujata, CISHB-2, Pant Aparna, Pant Shivani		
32. (*) (+)	Styler end cavity	Shallow	**3**	CISHB-1, NB-9, NB-17, Pant Aparna, Pant Sujata,	**C**	**VG**
		Depressed	**5**	NB-5, Pant Aparna,		

				Pant Shivani, CISHB-2, NB-16, Goma Yashi		
		Highly depressed	**7**	Pant Urvashi, NB-7		
33 (*)	Stem end Cavity	Shallow	**3**	CISHB-1, CISHB-2,NB-5, NB-16,	**C**	**VG**
		Depressed	**5**	Goma Yashi, NB-17, Pant Aparna, Pant Urvashi, Pant Sujata,		
		Flattened	**7**	NB-7		
34. (*) (+)	Seed Shape	Round	**3**	CISHB-1, NB-5, NB-16, Pant Sujata	**C**	**VG**
		Oblong	**7**	Pant Aparna, Pant Shivani,NB-9,CISHB-2,NB-17,Pant Urvashi, NB-7		
35 (*) (+)	Locule arrangement	Scattered	**3**	CISHB-2,NB-17	**C**	**VG**
		Centric	**5**	CISHB-1,Pant Aparna,NB-16,Pant Shivani,NB-9,		
		Highly centric	**7**	NB-5, Pant Shivani		
36 (*)	Fruit weight	Low<1.0 kg	**3**	NB-16, CISHB-1, Pant Sujata	**C**	**MS**
		Medium 1.0 - 2.0 kg	**5**	Goma Yashi, NB-5, Pant Aparna,		
		High >2.00 kg	**7**	NB-9, NB-17 CISHB-2, Pant Shivani, Pant Urvashi, NB-7		
	Crude Fiber (%)	Low	**1**	Goma Yashi, NB-5,	**C**	**MS**

(*)		Medium	**3**	Pant Sujata, Pant Aparna, NB-9, CISHB-2 CISHB-1.		
		High	**7**	NB-17, Pant Shivani, Pant Urvashi, NB-16		
38. (*)	Total number of Seeds	Low<125	**1**	Goma Yashi, Pant Urvashi	**C**	**MS**
		Mediums 126-175	**3**	NB-9, Pant Urvashi, NB-5,		
		High >175	**5**	Pant Sujata, CISHB-2, Pant Shivani , NB-7, NB-17, NB-16,CISHB-1		
39.	Total soluble solids (^{o}Brix) of pulp	Low <33 (^{o}Brix)	**3**	CISHB-1, CISHB-2, Pant Sujata,	**C**	**MS**
		Medium (34-38 ^{o}Brix)	**5**	Pant Aparna, NB-17, NB-7, NB-16,, NB-5		
		High (>38^{o}Brix)	**7**	Pant Shivani, Pant Urvashi, Goma Yashi		
40.	Total soluble solids(^{o}Brix) of mucilage	Low 40-45 (^{o}Brix)	**3**	Pant Aparna, CISHB-2, Goma Yashi,	**C**	**MS**
		Medium <46-50 (^{o}Brix)	**5**	NB-16, Pant Sujata, Pant Shivani		
		High>50 (^{o}Brix)	**7**	Pant Urvashi, NB-5,		

7. Explanation for the Table of Characteristics

Characteristic: 1. Growth habit



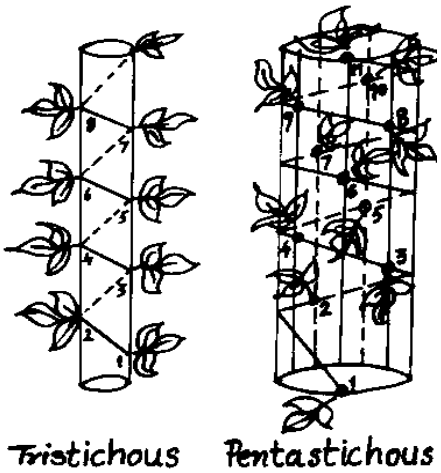

Drooping


Upright

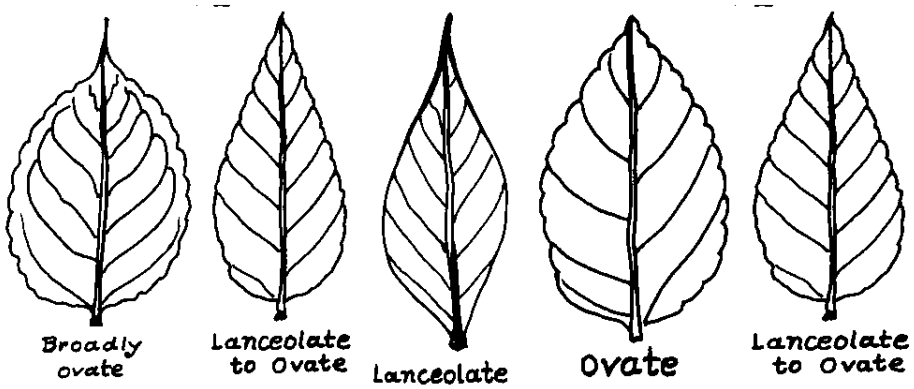

Spreading


Semi spreading

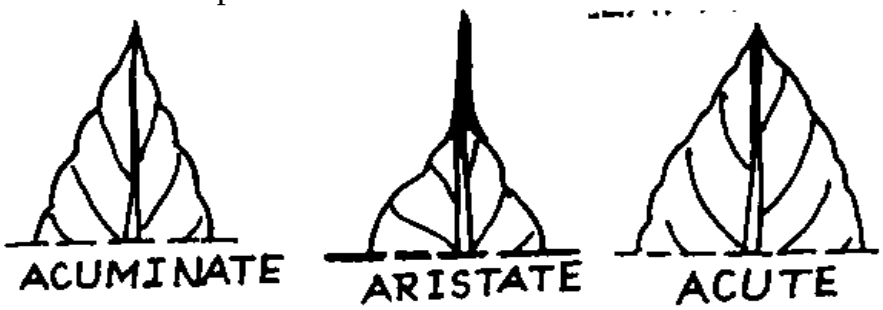
Characteristic: 3. Phyllotaxy



Characteristic: 6. Leaf shape

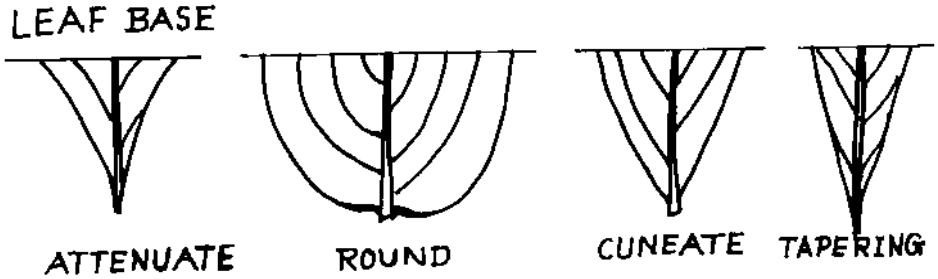


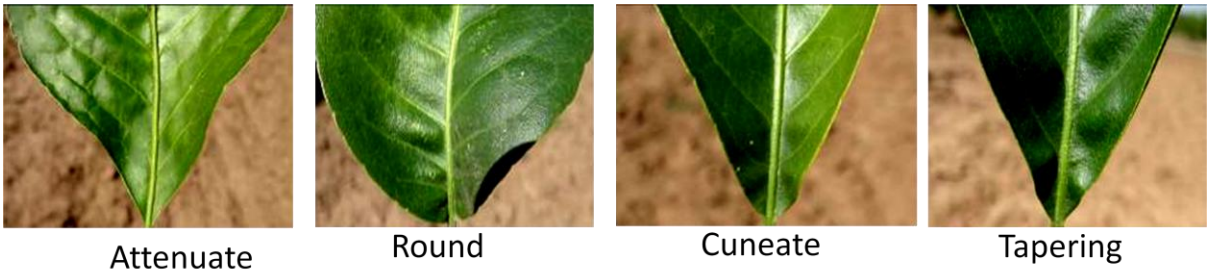
Characteristic: 7. Leaf apex



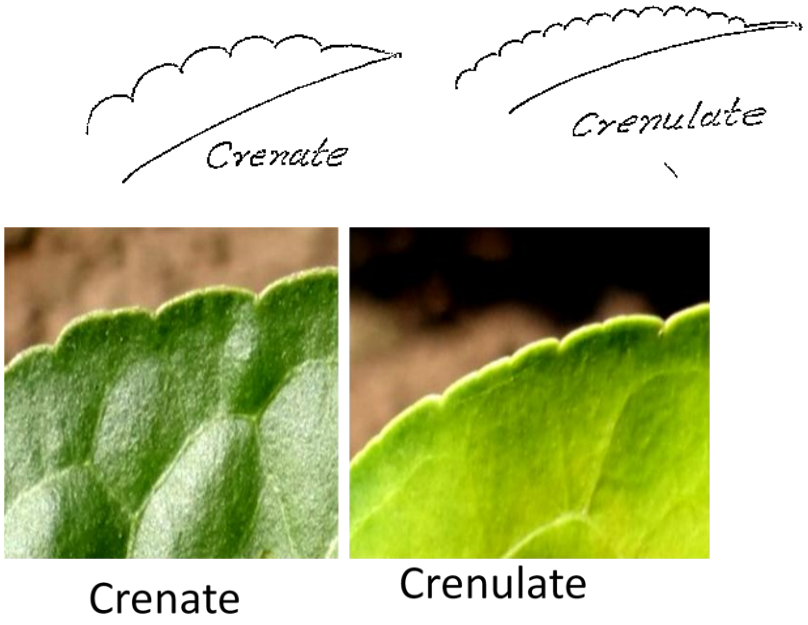
Acuminate Aristate Acute

Characteristic: 8. Leaf base



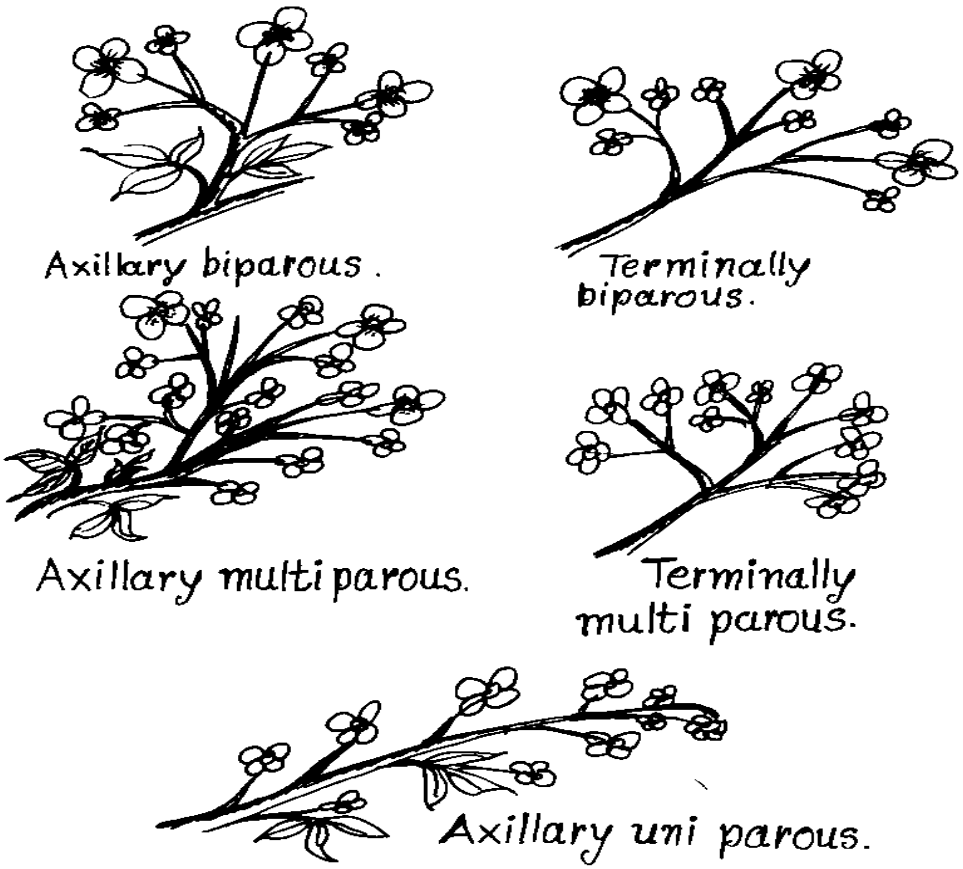


Characteristic: 15. Leaf margin



Characteristic: 16. Thorn

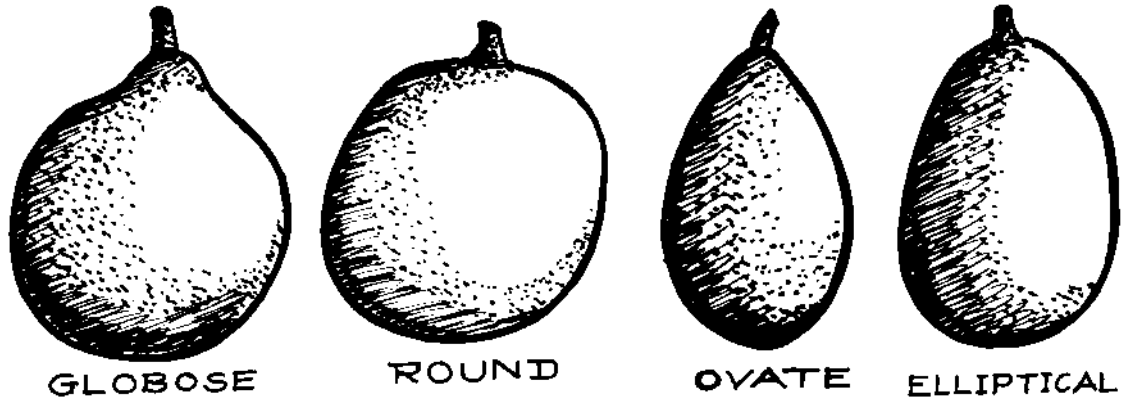
Characteristic: 17. Inflorescence type



Characteristic: 22. Mature fruit colour

Yellowish green | Greenish pale yellow | Green

Characteristic: 23. Fruit shape

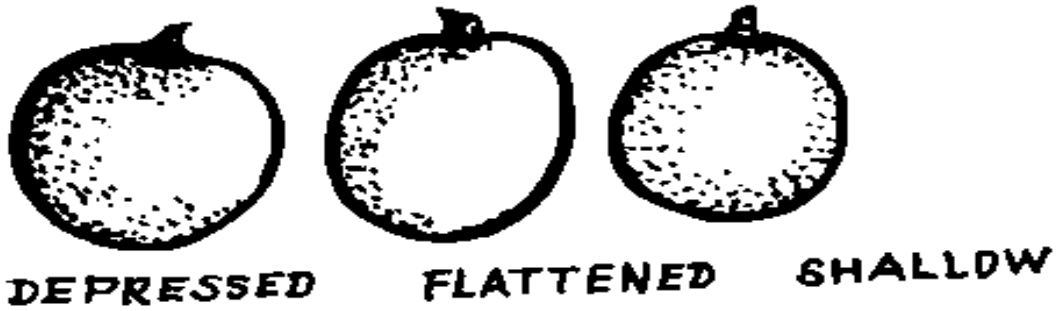


Globose Round Ovate Elliptical

Characteristic: 32. Styler end

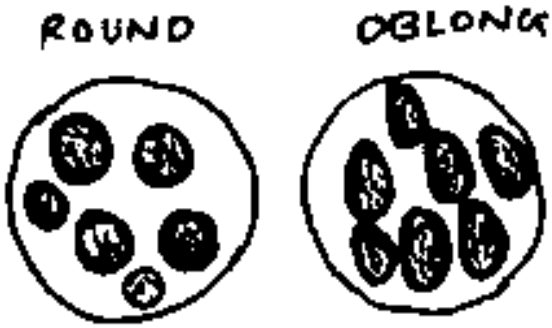
Depressed Highly depressed Shallow

Characteristic: 33. Stem end



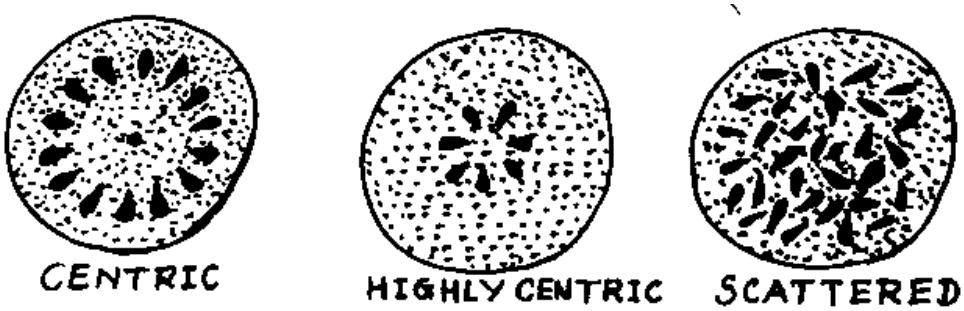
Depressed Flattened Shallow

Characteristic: 34. Seed shape



Round Oblong

Characteristic: 35. Locule arrangement



s

Characteristic 40: Fiber (%)

2g of moisture and fat free material was treated with 200 ml 0f 1.25% sulphuric acid (H_2SO_4). After filtration with Whattman paper no.4 and washing the residue was treated with 1.25% Na OH. It was filtered, washed with hot water and then 1% HNO_3 and again with hot water. The residue was ignited and the ash weighed. Loss in weight gave the weight of crude fiber.(Chopra and Kanwar,1991 and Mazumdar and Mazumdar, 2003)

$$\text{Crude Fiber\%} = \frac{(c-b)-(d-b)}{a} \times 100$$

a= wt. of sample

b= wt. of crucible

c= initial wt. of crucible containing tissue sample before ignition

d= final weight of crucible containing ash after ignition.

Characteristic 42 & 43: Pulp & mucilage TSS (^{0}Brix)

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 ^{0}Brix using hand-held/ digital refractometer (Krishna and Parashar, 2013). Similarly mucilage was collected from the fruit and directly measurement of TSS.

IX. Working Group details

The Test Guidelines developed by the Task Force (1/2011) constituted by the PPV&FR Authority.

The Members of the Task Force (14/2015)

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