

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

**INDIAN GOOSEBERRY
(*Emblica officinalis* Gaertn.)**



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

Indian gooseberry (*Emblica officinalis* Gaertn)

I. Subject

These test guidelines shall apply to all varieties and hybrids of Aonla (*Emblica officinalis* Gaertn.).

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 August-September shall be 07 (seven) 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 03-04 months from the date of grafting(propagated through grafting) 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).
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 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.
2. 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.

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.

2. 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 8m x 8m.

3. The additional test protocol for special purpose may be established by PPV & FRA.

4. ***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 (8x8m) 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 section7) 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 the mature fruit should be recorded at harvest maturity.
4. 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 and 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 *Aonla* varieties:

- a. Growth habit (Characteristic 2)
- b. Leaf: Shape (Characteristic 5)
- c. Inflorescence colour (Characteristic 10)
- d. Mature fruit: Shape (Characteristic 12)
- e. Mature fruit: Colour (Characteristic 13)
- f. Stone shape (Characteristic 18)

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 (I 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 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 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	State	Note	Example variety	Stage of observation	Type of assessment
1	2	3	4	5	6	7
1 (*)	Tree height	Dwarf	2	Narendra Aonla-6, Banarasi, Krishna, Chakaiya, Francis, Goma Aishwarya	A	VG
		Tall	1	Kanchan, Narendra Aonla -7, Anand-1, Anand-2 Narendra Aonla-10		
2 (*) (+)	Growth Habit	Erect	3	<u>NA-6,Chakaiya, Anand-1,Anand-2</u>	A	VG
		Spreading	5	<u>Narendra Aonla-10,NA-7, Francis, Goma Aishwarya</u>		
		Drooping	7	<u>Banarasi, Krishna, Kanchan,</u>		
3. (*)	Foliage	Sparse	3	Banarasi,Krishna, Chakaiya, Kanchan, Anand-1, Anand-2, NA-6	A	VG
		Dense	5	Francis, Narendra Aonla-10,Narendra Aonla-7, Goma Aishwarya		
4 (*)	Leaf size	Small (<1.25 cm)	1	Narendra Aonla-7,Krishna, Francis, Anand-1, Anand-2,	A	MS
		Large>1.35 cm	5	Chakaiya, Narendra Aonla-10,Narendra Aonla-7		
5 (+) (*)	Leaf shape	Elliptical	3	Narendra Aonla-7	A	VG
		Oblong	5	Chakaiya, Banarasi, Chakaiya, Narendra Aonla-10, Anand-1, Anand-2		
		Oval	7	Francis, Kanchan, Narendra Aonla-6, Goma Aishwarya		
6. (*) (+)	Leaf apex	Acute	1	Narendra Aonla-6, Chakaiya, Kanchan	A	VG
		Obtuse	7	Banarasi, Krishna, Francis, Narendra Aonla-7, Narendra Aonla-10, Anand-1, Anand-2		
7. (*)	Leaf Surface	Non glabrous	9	Narendra Aonla-7, Banarasi, Krishna	A	VG
		Glabrous	1	NarendraAonla-6, Kanchan, Francis		
8.	Trunk colour	Grey(197 A)	1	Chakaiya, Banarasi, Francis,	a	RHS

(*)				Anand-1, Anand-2		
		Whitish grey(199 B)	2	Narendra Aonla-7, Kanchan, Narendra Aonla-10, Goma Aishwarya, Krishna		
		Brownish grey(202 B)	3	Narendra Aonla-6		
9. (*)	Branchlet Colour	Deep red(181 A)	3	Banarsi	a	RHS
		Pinkish green(149A)	5	NarendraAonla-6		
		Yellowish green(144 B)	7	NarendraAonla-10		
10. (*) +	Inflorescence colour	Deep pink(47C)	3	Krishna, Banarasi, Narendra Aonla-10, NA-7	a	RHS
		Pinkish green(149 A)	5	NA-6, Chakaiya, Anand-2, Anand-1,		
		Yellowish green(147A)		Francis, Goma Aishwarya, Kanchan		
11(*)	Fruit surface	Smooth	1	Krishna, Goma Aishwarya, NA-7, Anand-2, Anand-1		
		Rough	9	NarendraAonla-10, Francis, Kanchan		
12. (+)(*)	Fruit Shape	Flattened Round	1	Chakaiya, Francis, Kanchan, NarendraAonla-10, Goma Aishwarya	a	VG
		Round	3	NarendraAonla-6		
		Triangular	5	Krishna, Banarasi		
		Oval	7	NarendraAonla-7, Anand-1, Anand-2		
13(*)	Fruit colour	Greenish(146A)	1	Anand-1, Anand-2, Banarasi,	a	RHS
		Yellowish green ithinkish tinge(144A)	3	NarendraAonla-7		
		Light green(145A)	5	NarendraAonla-6, Krishna, Francis, Chakaiya, NarendraAonla-10		
14.(*)	Fruit Stalk	Thick	1	Narendra Aonla-7, Banarasi, Krishna, NA-10	C	VG
		Thin	2	NA-6, Francis, Chakaiya, Kanchan, Anand-1		
15.(+)	Stem end	Flate	1	Krishna, NarendraAonla-7, NarendraAonla-10,	C	VG
		Depressed	2	Goma Aishwarya, Anand-2, Anand-1, Krishna		
16.(*)	Bearing tendency	Shy bearing		Banarasi, Krishna	C	VG
		Heavy bearing		Narendra Aonla-7, Anand-1, Goma Aishwarya		
17	Stone size	Small	3	Krishna, Kanchan, Chakaiya, Anand-1, Anand-2	C	MS
		Medium	5	NarendraAonla-10, Francis, Goma Aishwarya		
		Large	7	Chakaiya, Banarasi, Narendra Aonla -7		

18(+)(*)	Stone shape	Triangular	1	Krishna,Banra	a	VG
		Round	3	Kanchan ,Anand-1,Anand-2		
		Oval round	5	NA-7,Banarasi		
		Oval	7	NA-6, Francis,NA-10		
19(+)	Seed colour	Light Brown(177C)	3	Narendra Aonla-6, Goma Aishwarya	C	VG
		Dark Brown(177A)	7	Narendra Aonla-7,Banarasi		
20.(+)	Harvest Maturity	Early	1	Narendra Aonla-10, Banarasi, Krishna	D	VG
		Mid	5	NarendraAonla-7, Francis,Goma Aishwarya		
		Late	7	Chakaiya,NarendraAonla-6, Kanchan,		
21.	Fruit Weight	Low 30-40 gm	5	Narendra Aonla-10, Chakaiya, Francis	C	MS
		Medium 40-45gm	7	Banarasi,Goma Aaishwarya,		
		Very High >45gm	9	Krishna, Narendra Aonla-7		
22(*)	Fruit Segment	Six	1	NarendraAonla-6, NarendraAonla-10,Chakaiya,Anand-1,Anand-2 ,Banarasi,Kanchan,Francis,Goma Aishwarya	C	MS
		Six to Eight	2	Krishna, Narendra Aonla-7		
23(*)	Fruit Fibre (%)	Low fiber	3	Narendra Aonla-6, Krishna, Chakaiya, Goma Aishwarya,	C	VG
		High fiber	5	Kanchan, Francis, Anand-1, Anand-2		
24(*)	Pulp(%)	Low	1	Kanchan,Anand-2,NA-6	C	MS
		High	3	Narendra Aonla-6, Banarasi, NA-10		
25	Total Phenol content(TAEg/100g)	Low<1	1	Krishna, Banarasi,NA-6,NA-7,Anand-1,Banarasi, Narendra Aonla-7	C	MS
		High>1	7	Kanchan, Anand-2, Anand-1, Francis, Goma Aishwarya, Chakaiya,NA-10		
26	Vitamin C (mg/100g)	Low<400mg	1	Francis	C	MS
		Medium400-500mg	3	NA-4 NA-5 Chakaiya,		
		High>500mg	7	NA-10, Goma Aishwarya, Banarasi		

VIII. Explanation for the table of characteristics

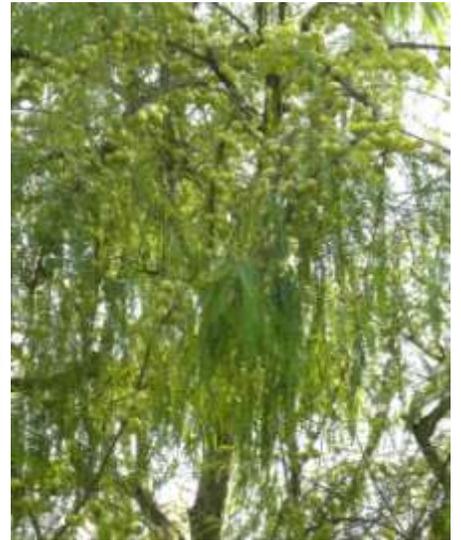
Characteristic 2: Growth Habit



Spreading



Erect



Drooping

Characteristic 5: Leaf Shape



Elliptical

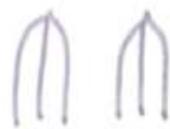


Oblong



Oval

Characteristic 6: Leaf Apex



Acute



Obtuse

Characteristic 14: Inflorescence Colour



Deep Pink

Pinkish Green

Characteristic 16: Fruit shape



Flattened round



Round



Triangular



Flattened oval

Characteristic 20 : Fruit stem end



Prominent



Less prominent

characteristic 24: Stone shape



Triangular



Round



Oval

Characteristic 32: Fiber(%)

2g of moisture and fat free material was treated with 200 ml of 1.25% sulphuric acid(H_2SO_4). After filtration with Whatman paper no.4 and washing the residue was treated with 1.25% NaOH. 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)

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

(a)

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 34: Pulp TSS ($^{\circ}$ 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 $^{\circ}$ Brix 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: Phenol content of pulp(mg/100g)

Reagents

i. Folin's reagent- 750 ml of water, add 100g of sodium tungstate, 20 g of phosphomolybdic acid and 50 ml of 85% phosphoric acid. Reflux the mixture for 2hr, cool to 25° C and dilute to 1000 ml with water. ii. Saturated sodium carbonate solution- To 100 ml of water, add 35 g of anhydrous sodium carbonate. Dissolve at $70-80^{\circ}$ C and cool overnight. Decant the clear liquid before use.

iii. Tannic acid std. solution- Dissolve 100mg of tannic acid in 1lt. of water. Prepare fresh solution for each determination. For estimation of total phenol content by tannic acid, took 5g sample and crush in 50 ml distilled water. Then 0.1 ml aqueous sample was taken in 25 ml volumetric flask. Add 1.25ml 1N Folin's reagent and 2.5 ml saturated sodium carbonate solution. Make up the volume by adding distilled water up to the mark of flask. Shake well and wait for 30mins for colour development. Then took optical density at 760nm on spectrophotometer. Ranganna (1986)

Characteristic 37: 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 (C₁₂H₆Cl₂NNaO₂.2H₂O) 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 formula-

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

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 aliquot 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 formula-

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

Litrature

Chopra, S L, Kanwar, J S . 1991. In: *Analytical Agricultural chemistry*, Vol.,IV .New Delhi, India, Kalyani Publications.P.297.

Krishna, H. and Parashar, A.2013. Phytochemical constituents and antioxidant activities of some Indian jujube (*Ziziphus mauritiana* Lamk.) cultivars. *Journal of Food Biochemistry*,doi:10.1111/jfbc.12008. (<http://onlinelibrary.wiley.com/doi/10.1111/jfbc.12008/abstract>)

Mazumdar, B.C and Mazundar K. 2003. *Methods on Physico-chemical Analysis of fruits*,University college of Agriculture, Calcutta University.108-109.

Ranganna, S. 1986. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*. 2nd ed. Tata McGraw-Hill, New Delhi, India. 1112 p.

IX. Working Group details

The Test Guidelines developed by the Task Force (08/2014) constituted by the PPV & FR Authority consultation with the Nodal officer, Dr. Devendra Pandey, Pr. Scientist, CISH, Lucknow and Co-Nodal Officer, Dr. A. K. Singh, Pr. Scientist, CISH, Lucknow.

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X. DUS Test Centres

Nodal Centre	Co Nodal centre
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