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

On

Acid lime

(*Citrus aurantifolia* Swingle.)

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

Acid lime (*Citrus aurantifolia* Swingle)

I. Subject

These test guidelines shall apply to all the varieties of acid lime (*Citrus aurantifolia* Swingle)

II. Materials required

1. The Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) shall decide on the quantity and quality of the planting materials required for testing the varieties and where it is to be delivered for registration under the Protection of Plant Varieties and Farmers' Rights (PPV & FR) Act, 2001.
2. Applicants submitting such materials from a country other than India shall make sure that all customs and pre and post quarantine requirements stipulated under relevant national legislations and regulation are complied with.
3. The materials are to be raised as nucellar seedlings and a minimum of ten plants to be supplied by the applicants or his/her nominee during the month of June- July for each DUS Centre. Planting materials supplied shall be healthy and free from pests, diseases and mechanical injury. Age of the plants shall be above six months from the date of transplanting in secondary nursery and raised in the black polythene bags 300 µ thickness UV stabilized (12cm x 6cm size) with potting mixture (soil, FYM and sand in 1 : 1: 1 ratio).
4. The plants should not have undergone any treatment which would affect the expression of the characters of the variety, unless the competent authority allows or requests for any such treatment.
5. The planting material shall not have undergone any chemical and bio-physical treatment unless the competent authority or applicant specifically request for such treatment. If it has been treated, full details of the treatment must be mentioned explicitly.

III. Conduct of test

1. The minimum duration of the DUS tests shall normally be at least for two independent identical fruiting seasons in different years.
2. The test should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination. In particular, it is essential that the tree produces a satisfactory crop of fruit in each of the fruiting seasons in two consecutive years. In case of any climatic vagaries data from third fruiting season may also be considered.

3. Test Design

The design of the tests should be such that plants or parts of the plant may be removed for measurement or counting without prejudice to the observations which may be made up to the end of the vegetative /fruiting season as the case maybe. Unless otherwise indicated, all observations are to be recorded on five plants**.**

Additional Tests

Additional tests, for examining special characteristics, may be established by the PPV&FR Authority.

4. On- site testing :

The guidelines developed by PPV&FR Authority for on- site testing will be followed with the specific requirement for acidlime.

- The age of the plants for on-site shall be minimum of five years from the date of planting in the field.
- A minimum of two plants must be made available for field gene bank. For inspection and examination even single tree could be considered only for farmers' varieties. The trees should be healthy, free from pests and diseases and raised under standard management practices.
- On-site examination shallbe arranged during vegetative and fruiting seasons.

IV. Methods and observations

i. The characteristics described in the Table of Characteristics (see section VII) shall be used for the testing of candidate varieties.

ii. For the assessment of DUS characters, observations shall be made on five plants.

Observations

(a) Leaf: Observations on the leaf should be made on the fully expended leaves of spring flush.

(b) Fruit: Observations on the fruit should be made at the stage of harvest maturity. Fruits should be sampled from the periphery of the tree

(c) Fruit rind: Observations on the fruit rind should be made at the middle, between the base and apex of the fruit.

(d) Number of spines per 30 cm length from basal bud on one year old shoot.

V. Grouping of varieties

1. The candidate varieties of 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 in which their various states are fairly evenly distributed across all the varieties in the collection are suitable for grouping purpose.
2. Characteristics for grouping are those in which the documented states of expression, even when produced at different locations. These can be used, either individually or in combination

with other such characteristics to (a) select varieties of common knowledge that can be excluded from the growing trials used for examination of distinctiveness; and (b) organize the growing trials so that similar varieties are grouped together.

The following characteristics are to be used for grouping of acid lime varieties:

(a) Tree growth habit (characteristic - 1)
(b) Spine density (characteristic - 2)
(c) Fruit diameter (characteristic - 8)

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 in all the varieties and shall always be included in the description of the variety. In exceptional cases wherein the state of expression of any of these characters is not recorded due to environmental vagaries, adequate explanation may 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 are to be taken are given in the explanation or figure (s) for clarity and not the colour variation.

4. A code number given in the sixth column of Table of characteristics indicates the optimum stage for the observation of each characteristics during the growth and development ofthe plant. The relevant growth stages corresponding to these code numbers are described below:

Code for the growth stages :

Growth stage	**Code**
Full grown bearing tree	100
One year old spring flush shoots	30
Fully expanded leaves of spring flush shoots	30
Harvest maturity	95

(a) Observations on fully expanded leaf on the middle portion of the spring flush.

(b) The mature/ripe fruit is the fruit at the stage ready for consumption. This stage is reached when the segment is juicy and fruits developed characteristic colour.

(c) The colour expression must be recorded using RHS colour chart

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 plant
MS : Measurement of a number of individual plants or parts of plant
VG : Visualassessment by a single observation of a group of plants or parts of plant
VS : Visualassessment by observation of individual plants or parts of plant

VII. Table of Characteristics

S. No	Characteristics	States	Note	Example varieties	Stage of observation (Code)	Type of assessment
1 (*) (+)	Tree growth habit	Erect	1	Chakradhar	Full grown bearing tree (100)	VG
		Spreading	2	Sai Sharbati, Vikram, Pramalini, Balaji,Phule Sharbati.		
		Drooping	3	-		
2. (*)	Spine density on the adult tree (No. of spines on one year old spring shoot, 30cm length)	Low (< 10)	3	Chakradhar	Full grown bearing tree (100)	MG
		Medium (10-15)	5	Sai Sharbati, Vikram, Pramalini, Balaji, Phule Sharbati		
		High (>15)	7	--		
3.	Spine length (mm)	<5	1	Chakradhar	One year old spring flush shoots (30)	MS
		5 -15	2	Sai Sharbati, Vikram, Pramalini, Balaji, Phule Sharbati		
		>15	3	--		
4 (+)	Leaf lamina length [mm]	Short(<60)	3	Chakradhar	Fully expanded spring flush leaves (30)	MG
		Medium(60-70)	5	Sai Sharbati, Vikram, Pramalini, Balaji, Phule Sharbati,		
		Long(>70)	7	--		
5. (+)	Leaf lamina width [mm]	Narrow(<35)	3	Chakradhar	Fully expanded spring flush leaves (30)	MG
		Medium(35 - 40)	5	Sai Sharbati, Vikram, Pramalini, Balaji, Phule Sharbati,		
		Broad(>40)	7	--		
6. (+)	Petiole wings	Absent	1	Chakradhar	Fully expanded spring flush leaves (30)	VG
		Present	9	Sai Sharbati, Vikram, Pramalini, Balaji, Phule Sharbati		

7.	Fruit weight (g)	Light (<40)	1	Vikram, Pramalini, Chakradhar	Harvest maturity (95)	MG
		Heavy (>41)	2	Sai Sharbati, Balaji, Phule Sharbati		
8 (*) (+)	Fruit diameter (mm)	Small (<40)	3	Chakradhar,	Harvest maturity (95)	MG
		Medium (41 - 45)	5	Vikram, Pramalini, Balaji		
		Large(>45)	7	Sai Sharbati, Balaji, Phule Sharbati		
9 (+)	Fruit length (mm)	Short (<40)	3	Chakradhar	Harvest maturity (95)	MG
		Medium (40 - 45)	5	Vikram, Pramalini, Balaji		
		Long(>45)	7	Sai Sharbati, Phule Sharbati		
10	Albedo colour	Greenish	1	--	Harvest maturity (95)	VS
		White	2	Sai Sharbati, Vikram, Pramalini, Balaji,Chakradhar, Phule Sharbati		
		Yellow	3	---		
11	Fruit axis	Solid	1	Sai Sharbati, Vikram, Pramalini, Balaji, Phule Sharbati	Harvest maturity (95)	VS
		Hollow	2	Chakradhar		
12	Number of segments per fruit	8-10	1	Sai Sharbai, Vikram, Pramalini, Balaji,Chakradhar, Phule Sharbati	Harvest maturity (95)	VS
		>10	2	--		
13	Fruit rind (epicarp) thickness (mm)	Thin (<2)	3	Sai Sharbati, Vikram, Pramalini, , Balaji,Chakradhar, Phule Sharbati	Harvest maturity (95)	MS
		Thick(>2)	5	--		
14	Fruit juiciness (%)	Low (<40)	3	--	Harvest maturity (95)	MS
		Medium (40 to 50)	5	Vikram, Pramalini, Chakradhar		
		High (>50)	7	Sai Sharbati, , Balaji, ,Phule Sharbati,		
15 (+)	Total Soluble Solids (TSS, ^{0}Brix)	Low (<6)	3	-	Harvest maturity (95)	MS
		Medium (6 to7)	5	--		
		High (>7)	7	Sai Sharbati, Vikram, Pramalini, Balaji,Chakradhar ,		

				Phule Sharbati		
16 (+)	Titratable acidity (% citric acid)	Low (<5)	3	--	Harvest maturity (95)	MS
		Medium (5 to 6)	5	Chakradhar		
		High (>6)	7	Sai Sharbati, Vikram, Pramalini, Balaji, Phule Sharbati		
17	Seediness (Number of seeds/ fruit)	<4	1	Chakradhar	Harvest maturity (95)	MS
		4-10	2	Sai Sharbati, Vikram, Pramalini, Balaji, Phule Sharbati, Niboo		
		>10	3	--		

VIII. Explanation on the Table of Characteristics :

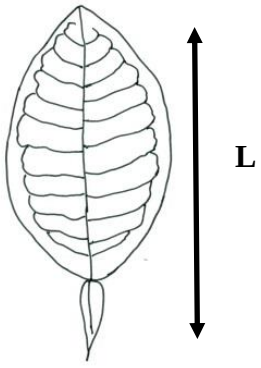
Characteristic 1. Tree growth habit

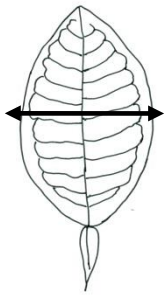
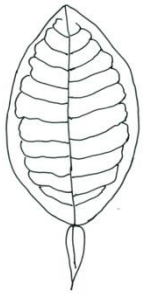
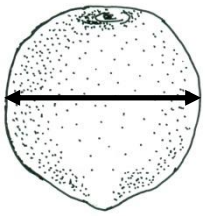
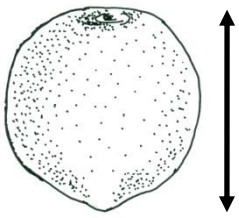
Recorded on the tree not less than 5years age in natural state just after fruit harvesting

1
Erect

2
Spreading

Characteristic 4. Leaf lamina length [mm] Recorded from petiole base to lamina tip



Characteristic 5. Leaf lamina width [mm]	
 **W**	
Characteristic 6. Absence/presence of petiole wings	
	
1 **Absent**	**2** **Present**
Characteristic 8. Fruit diameter	
 **D**	
Characteristic 9. Fruit length	
 **L**	

Characteristic 16. Fruit juice TSS **(^{0}Brix)**

The fruit samples were harvested as per maturity standard. The juice will be extracted by juicer or electronic juicer machine and total soluble solids **(**TSS) determined. The hand held/ digital refractometer should be used to measure the TSS ^{0}brix in juice sample. One or two

drops of the juice should be placed on refractometer and per cent TSS on the scale should be recorded. The reading are to be taken at room temperatures.

Characteristic 17. Titratableacidity (% Citric acid)

The juice acid content of the samples should be recorded by visual titration method as suggested by Ranganna(1986). The titration sample prepared with 5ml of juice mixed with 20 ml of distilled water put in volumetric flask to make up the volume to 25 ml. Thereafter 5 ml mixed sample should be taken for further titration using phenolphthalein as an indicator against 0.1 N sodium hydroxide. The titrated acidity expressed as percentage citric acid.

$$\text{Acidity (\%)} = \frac{\text{Titre value x Normality of alkali x Volume made up x Equivalent weight of acid (i.e. 64 x100)}}{\text{Volume of aliquottaken for estimation x Weight or volume of sample taken x1000}}$$

IX. Literature :

- **IPGRI. (1999).** Descriptors for Citrus. International Plant Genetic Resources Institute, Rome, Italy, pp.66.
- **Ranganna, S.** (**1986).** Manual for Analysis of Fruit and Vegetable Products. Tata McGraw Hill Pub. Co., New Delhi.
- **Singh, I.P. and Singh, Shyam (2003)**. Exploration, Collection and Mapping of Citrus Genetic Diversity in India. *Technical Bulletin No. 7*, NRC for Citrus, Nagpur, Maharashtra, pp.230.
- **Singh, I.P. and Singh, Shyam (2006)**. Catalogue of Citrus Germplasm. *Catalogue No. 1*, NRCC, Nagpur, pp.56.
- **Singh, I.P. and Singh, Shyam (2006).** Citrus Monograph. *Monograph No.1*, NRCC, Nagpur, pp.96.
- **Singh, I.P. (2013).**Community Fruits Catalogue of Amaravati Site: Citrus and Mango Diversity.*Fruits Catalogue No. - 2*.NRC for Citrus, Amravati Road, Nagpur, Maharashtra, pp.27.

X. Working Group details

The Test Guidelines developed by the NRC for Citrus, Nagpur was finalized by the Task Force (1/2013) constituted by the PPV & FR Authority.

The Members of the Task Force (1/2013)

Dr. V. A. Parthasarathy - Chairman
Dr B.M.C. Reddy - Member
Dr S. N. Pandey - Member
Dr H. Ravishankar - Member
Dr Umesh Srivastava - Member
Dr I. P. Singh - Member
Dr. Tejbir Singh - Member Secretary

Nodal Officer

Dr I.P. Singh, Principal Scientist (Hort.) and Nodal officer DUS project

National Research Centre for Citrus (NRCC), Amravati Road, Nagpur

Co-Nodal Officer

Dr R.K. Sonkar, Principal Scientist (Hort.)

National Research Centre for Citrus (NRCC), Amaravati Road, Nagpur

IX. DUS testing centers

Nodal DUS Test Centre	**Other DUS Test Centres**
National Research Centre for Citrus (NRCC), Amravati Road, Nagpur (Maharashtra)-440010	Horticultural Experiment Station, Indi /Bijapur, Karnataka.