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

CASSAVA

(*Manihot esculenta* Crantz.)

PROTECTION OF PLANT VARIETIES AND FARMERS' RIGHTS AUTHORITY

(PPV&FRA)

Government of India

CONTENTS

CASSAVA

(*Manihot esculenta* Crantz)

Cassava (*Manihot esculenta* Crantz) is one of the most important tropical root and tuber crops and is grown for it starchy tuberous roots. It belongs to the family Euphorbiaceae. According to the prevailing knowledge, cultivated cassava originated in South America and was domesticated less than 10,000 years ago with evidence of ancient cultivation in Brazil, Peru, Columbia and Venezuela. Cassava was introduced to India by the Portuguese when they landed in the Malabar region, presently part of the Kerala state, during the 17^{th} century, from Brazil. Cassava is an out breeding species possessing 2n = 36 chromosomes and is considered to be an amphi-diploid or sequential allo-polyploid. Triploid [(2n = 54) and (2n = 72)] hybrids are also available in cassava. It is an erect growing shrub, branched or un-branched with palmate partite leaves. The cassava plant is monoecious, protogynous and bear separate male and female flowers on the same plant. The improved varieties were mainly developed through hybridization followed by clonal selection. Besides hybrids, several landraces were also released for cultivation through clonal selection. Most of the released varieties are diploid in nature.

Cassava cultivation in India is mostly confined to Kerala, Tamil Nadu, Andhra Pradesh and North-Eastern hill region. Considering its nature as an industrial raw material for starch production, cassava cultivation is catching up in non-traditional areas like Maharashtra and Gujarat. In Kerala, it is grown both under upland as well as under low land conditions. It is also raised as an inter crop in coconut gardens under homestead farming situation. In Tamil Nadu, the crop is cultivated in plains and hills. Tamil Nadu is the only place in the world where cassava is raised under irrigation in plains. Under hills, it is purely rainfed and major portion of production goes for industrial use (Edison *et al*., 2006). Intercropping of pulses and vegetables is also practiced. In Andhra Pradesh, the crop is cultivated under rainfed conditions and is used for starch extraction. In the NEH region, cassava is grown under mixed stand in homesteads and mostly used for human consumption and as feed of pigs, particularly in Tripura.

I. Subject

These test guidelines shall apply to all varieties, hybrids and parental lines of Cassava (*Manihot esculenta* Crantz.).

II. Planting material required

1. The Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA) shall decide when, where and in what quantity and quality the seed/planting material is required for testing the variety denomination applied for registration under the Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act, 2001. Applicants submitting such planting 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 material is to be supplied in the form of cuttings taken immediately (e.g., not later than 15 days, during May-June before onset of south west monsoon in India) after harvest.

2. The minimum quantity of planting material to be supplied by the applicant in one or several samples should be enough for making 100 cuttings for each centre, each one with a length of 20 cm with minimum 5 to 8 viable buds. The diameter of the stem cuttings to be used should be around 2-3 cm. Cuttings should be taken from the middle part of the stem of a 6-10 months old plant.

3. The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

4. The planting material should not have undergone any chemical or bio-physical treatment which could affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

III. Conduct of tests

1. The minimum duration of DUS tests shall normally be at least two independent similar growing seasons with two consecutive plantings, the second being a replanting with plant material retrieved from the first crop.

2. The test shall normally be conducted at least at two locations. If 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 (a third location) or under special test protocol on a expressed request of the applicant.

3. The field tests shall be carried out under conditions (irrigated/rainfed) favouring normal growth and expression of all test characteristics. Each test shall include about 75 plants and planting space specified below across three replications. Separate plots for

observation and for measurement can only be used, if they have been subjected to similar environmental conditions. All the replications shall have similar environmental conditions of the test location.

4. The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

5. **Test plot design:**

No. of rows	:	5
Row length	:	4.5 m
Plant to plant spacing	:	90 cm
Row to row spacing	:	90cm
Plants/replication	:	25
Number of replications	:	3

6. Observations should not be recorded on the plants in border rows.

7. Additional test protocols for special tests 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 for their DUS test.

2. For the assessment of Distinctiveness and Stability, observations shall be made on at least 30 plants or parts of 30 plants, which shall be equally divided among three replications (10 plants per replication).

3. For the assessment of Uniformity, a population standard of 1% and an acceptance probability of at least 95% shall be applied. The number of off types shall not exceed 2 plants out of 100 plants.

4. For the assessment of all colour characteristics, the latest Royal Horticultural Society (RHS) colour chart shall be used.

5. Unless otherwise indicated, all observations on the plant, the leaf and the stem should be made before the end of the growing phase, during the full expression time. Unless otherwise indicated, all observations on the shoot shall be made on the main shoot (the tallest).

6. All observations on the tubers should be made at the harvestable maturity.

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 purposes.

2. The following characteristics shall be used for grouping of cassava varieties:

i. Pubescence on apical leaves (Characteristic 1)

ii. Predominent shape of central leaf lobe (Characteristic 3)

iii. Petiole colour (Characteristic 4)

iv. Colour of mature stem: exterior (Characteristic 15)

v. Tuber rind colour (Characteristic 28)

vi. Tuber flesh colour (Characteristic 29)

VI. Characteristics and symbols

1. To assess Distinctiveness, Uniformity and Stability(DUS), the characteristics and their states as given in the Table of characteristics (Section VII) shall be used.

2. States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Notes (1 to 9) shall be used to describe the state of each character for the purpose of digital data processing and these notes shall be given against the states of each characteristic. In the case of qualitative and pseudo-qualitative characteristics, all relevant states of expression are presented in the characteristic.

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 explanations 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 for the colour variation.

1. The optimum stage (Months after planting) of plant growth for assessment of each characteristic is given in the column 6 of Table of Characteristic (Section VII)

2. 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 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 observations of individual plants or parts of plants 6

VII. Table of characteristics

Sl. No.	Characteristic	States	Notes	Example varieties	Stages of observation (months)	Type of assessment
1. (*) (+)	Pubescence on apical leaves	Glabrous	1	Sree Sahya, Sree Visakham	03	VS
		Moderate	5	H-97, H-165		
		High	7	H-226		
2. (*) (+)	Colour of first fully expanded leaf	Light green (Yellow green group 144)	3	H-226	03	VS
		Dark green (Green group 139)	5	Sree Harsha		
		Purplish green	7	IPS-3		
		Purple (Purple group N77)	9	IPS2-1		
3. (*) (+)	Predominant shape of central leaf lobe	Linear	1	CI-1108	06	VG
		Pandurate	3	CR 43-7, CI 1616		
		Lanceolate	5	Vellayani Hrazwa, Sree Vijaya		
		Elliptic-Lanceolate	6	Sree Jaya		
		Obovate-Lanceolate	7	Sree Harsha, 9S 242		
		Ovoid	9	Trl-17, Sree Apoorva		
4. (*) (+)	Petiole colour	Yellowish green	1	H-226	06	VS
		Green	2	IMS2-8		
		Reddish cream	4	Co-4		
		Reddish-green	5	Sree Jaya		
		Greenish-red	6	CI-1008		
		Red	7	Sree Apoorva		
		Purple	8	IPS2-1		

5. (*) (+)	Mature leaf color (dorsal)	Light green (Green group 143)	1	Vellayani Hrazwa	06	VS
		Dark green (Green group N137)	5	8S501-1		
		Purplish green (Purple group N77)	9	IPS2-1, Aromal		
6. (+)	Predominant number of leaf lobes	Three lobed	3	IH-5/21	06	VG
		Five lobed	5	Co-3		
		Seven lobed	7	Sree Sahya, Sree Harsha		
		Nine lobed	9	Sree Athulya, Sree Apoorva		
7. (+)	Length of central leaf lobe (cm)	Short(<10)	3	Co-2	06	MS
		Medium(10-15)	5	Sree Sahya, Sree Rekha		
		Long(>15)	7	Sree Athulya, Sree Apoorva		
8. (*) (+)	Width of central leaf lobe (cm) (at max. width)	Narrow(>2)	3	Sree Reksha-2	06	MS
		Medium(2-3)	5	Sree Visakham		
		Broad(>3)	7	Sree Athulya		
9. (+)	Leaf lobe margin	Smooth	3	Sree Rekha, Sree Prabha	06	VG
		Winding	5	Sree Sahya, Sree Harsha		
10. (*)	Petiole length (cm)	Short (< 25)	3	Sree Prabha, Co-2	06	MS
		Medium (25-35)	5	H-97		
		Long (>35 cm)	7	Sree Apoorva, Sree Athulya		
11. (*) (+)	Leaf vein colour	Green (Yellow green group 144)	3	Sree Visakham, H-226	06	VS
		Partly reddish (Red purple group 64)	5	Kalpaka		
		All red (Red purple group 64)	7	Aromal		

12. (*) (+)	Orientation of petiole	Inclined upwards	1	H-97	06	VG
		Horizontal	3	Sree Sahya		
		Inclined downwards	5	Sree Prakash		
		Irregular	7	Sree Pavithra		
13. (*) (+)	Young stem colour (at top 20 cm of plant)	Light green (Yellow green group N144)	1	Sree Vijaya	06	VS
		Dark green (Green group 143)	2	H-226		
		Greenish red	4	Sree Swarna		
		Greenish purple (purple group N77+ Green group 144)	5	IPS2-1, IPS-3		
14. (+)	Prominence of foliage scars	Semi-prominent	3	Vellayani Hrazwa	06	VG
		Prominent	5	Sree Athulya		
		Highly prominent	7	Sree Sahya, MVD-1		
15. (*) (+) .	Colour of mature stem: exterior	Silver	1	CR54A-3, Sree Rekha	09	VS
		Grey	2	M 4		
		Orange	3	9S-139		
		Light brown	4	Sree Apoorva		
		Dark brown (Brown group 200B)	5	8S56		
		Golden-red	7	Sree Swarna		
16 (*) (+)	Stem: distance between leaf scars	Short (≤3.0)	3	Co-3, Vellayani Hrazwa	06	MS
		Medium(3.1-6.0)	5	Sree Athulya		
		Long(≥6.1)	7	11S55		
17. (*)	Growth habit of Stem	Straight	3	Sree Rekha	06	VG
		Zig zag	7	CI-1162		
18. (*) (+)	Plant type	Erect	1	Sree Prakash, H-97	09	VG
		Semi-spreading	3	Sree Prabha		
		Spreading	5	Tr 11-4		
19.	Plant height (cm)	Short (<100)	3	16-5	09	MS

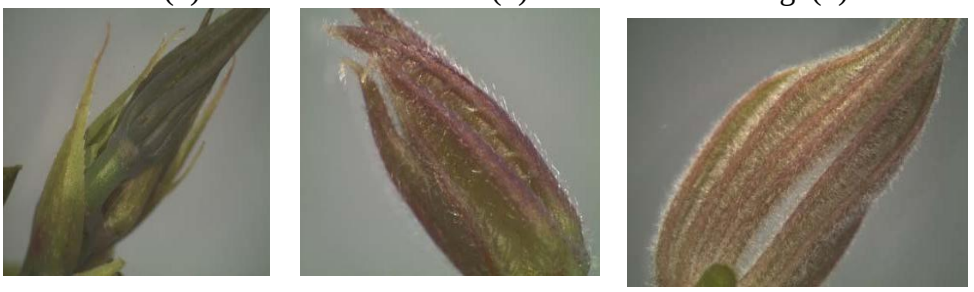
(*) (+)		Medium (100-200)	5	Vellayani Hrazwa		
		Tall (>200)	7	Sree Athulya, Sree Visakham		
20. (*) (+)	Plant branching Habit	Single	1	M4, Sree Prakash	09	VG
		Dichotomous	2	CI-1112		
		Trichotomous	3	Tr-14		
		Tetrachotomous	4	9S172		
21.	Plant canopy	Compact	1	Tr21-11	06	VG
		Open	2	T 14-1		
22.	Fruit	Absent	1	Sree reksha I,II	09	VG
		Present	9	Tr 5-14		
23. (*) (+)	Crop maturity (in months)	Early (<6 months)	3	Vellayani Hrazwa	06/09	MG
		Medium (6-8 months)	6	Sree Prakash		
		Late (>8 months)	9	Sree Sahya		
24. (*)	Tuber peduncle	Absent	1	Sree Vijaya	09	VG
		Present	9	Sree Sahya		
25. (+)	Tuber shape	Conical	1	Vellayani Hrazwa, 9S132	09	VG
		Conico-cylindrical	2	9S127		
		Cylindrical	3	Sree Visakham		
		Irregular	4	CI-1004		
26. (*) (+)	Tuber peelability	Easy	1	Vellayani Hrazwa	09	VG
		Medium	5	9S 142		
		Difficult	7	Sree Prabha		
27. (+)	Tuber skin colour	Cream	1	H-226	09	VS
		Light brown	3	H165		
		Reddish brown	5	Sree Swarna		
		Dark brown	7	Co-1		
28. (*)	Tuber rind colour	Cream	1	11S-55	09	VS
		Cream with pink patches	3	Sree Padmanbha,IPS 2-1		
		Yellow	5	H-97		
		Pink	7	Sree Swarna		

(+)		Purple	9	I-PS2-1		
29. (*)	Tuber flesh colour	White	1	M4	09	VS
		Cream	2	Sree Prabha		
		Light yellow	3	Sree Vijaya		
		Dark yellow	4	11S27		
30. (+)	Mealiness of cooked tuber	Fairly mealy	1	Sree Harsha	09	VG
		Moderately mealy	2	Sree Swarna		
		Very Mealy	3	M4, Vellayani Hrazwa		

VIII Explanation for the table of characteristics

Characteristic 1. Pubescence on apical leaves: Record the most frequent occurrence from the leaf taken from a mid-height position during active vegetative growth.

Glabrous(1) Moderate(5) High(7)



Characteristic 2. Colour of first fully expanded leaf: Record the most frequent occurrence at three months after planting.

Light green(3)	Dark Green(5)	Purplish green(7)	Purple(9)
			

Characteristic 3. Predominent shape of central leaf lobe: Record the most frequent occurrence from the leaf taken from a mid-height position during active vegetative growth.

Linear(1)

Pandurate(3)

Lanceolate(5)

Elliptic lanceolate(6)

Obovate lanceolate(7)

Ovoid(7)

Characteristic 4. Petiole Colour: Record the most frequent occurrence from leaf taken from mid height position during active vegetative growt

Yellowish Green (1)	Green(2)	Reddish Cream(3)	Reddish Green(5)	Greenish Red(6)	Red(7)	Purple(8)

Characteristic 5. Mature Leaf colour (dorsal): Observe a leaf from the middle of the plant and record the most frequent occurrence. No intermediates allowed.

Light Green (1)

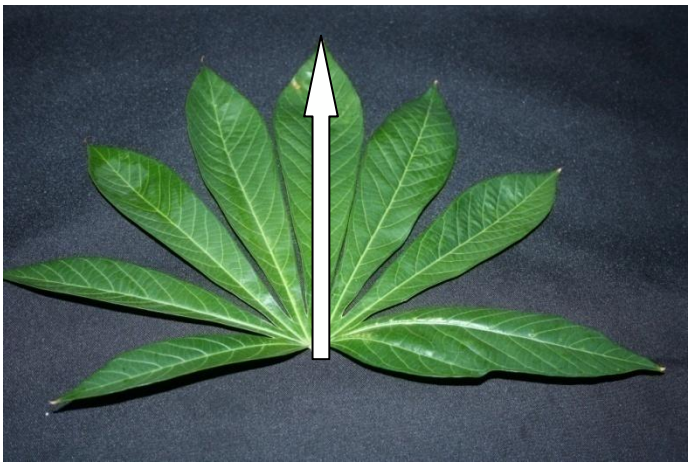
Dark Green(5)

Purplish Green(9)

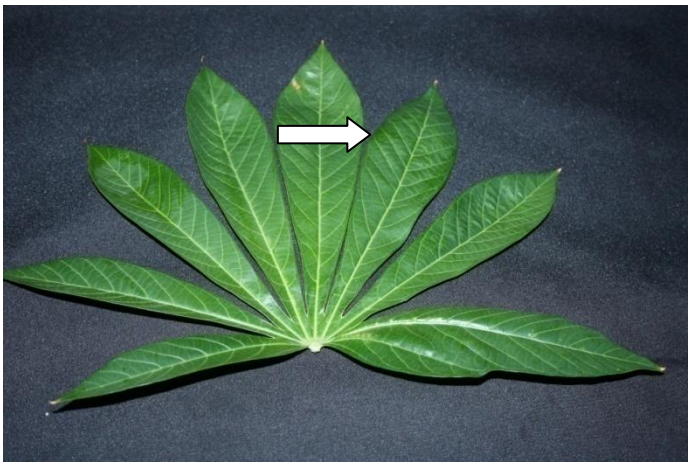
Characteristic 6. Predominant number of leaf lobes: Observe a leaf from the middle of the plant. Assess on five leaves and take the predominant number of lobes during active vegetative growth . Record only one score.

3. Three lobed	5. Five lobed	7. Seven lobed	9. Nine lobed

Characteristic 7. Length of central leaf lobe (cm): Measure three leaves from the middle of the plant. Measure from the intersection of all lobes to the end of the middle lobe.



Characteristic 8. Width of central leaf lobe (cm): Measure three leaves from the middle of the plant. Measure from the widest part of the middle lobe.



Characteristic 9. Leaf lobe margin: Observe from the mid-height of the plant. Record the most frequent occurrence during active vegetative growth.

Characteristic 10. Petiole length (cm): Observe from the mid-height of the plant during active vegetative growth. Measure three leaves/ plant.

Characteristic 11. Leaf vein colour: Observe near the base of the lobes, on the upper/dorsal side of the leaf, on the central lobe from a leaf from the mid-height of the plant. Record the most frequent occurrence.

Green(3)

Partly reddish (5)

All red(7)

Characteristics 12. Orientation of petiole: Observe at the middle of the plant. Take a general observation across the row.

Inclined upwards(1)

Horizontal(3)

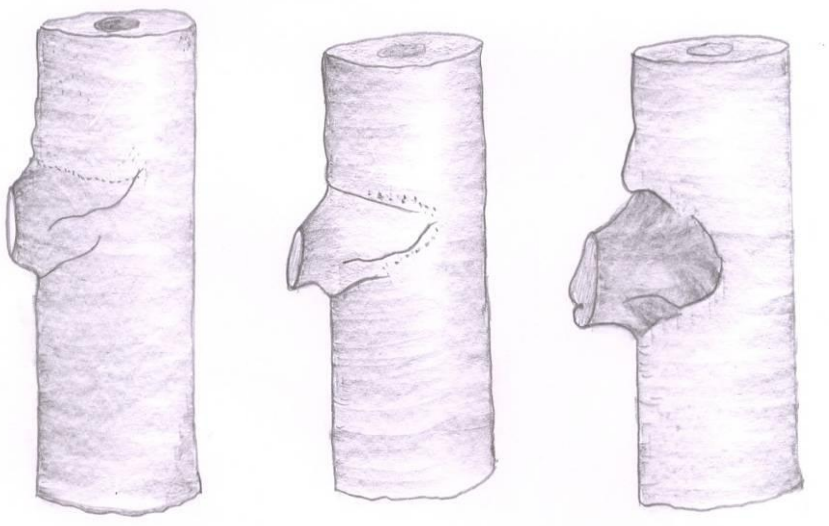
Inclined downwards(5)

Irregular(7)

Characteristic 13. Young stem colour (at top 20 cm of plant): Observation to be taken on top 20 cm of plant. Record the most frequent occurrence during active vegetative growth .

Light green(1)	Dark Green(2)	Greenish red(4)	Greenish purple(5)
			

Characteristic 14. Prominence of foliage scars: Observe from the middle third from the top of the plant. Record the most frequent occurrence during active vegetative growth.



Semi prominent(3)	Prominent(5)	Highly prominent(7)

Characteristic 15. Colour of mature stem- exterior: Observe from the middle third from the top of plant during active vegetative growth (09).

Silver(1)

Grey(2)

Orange(3)

Light brown(4)

Dark brown(5)

Golden red(7)

Characteristic 16. Stem:distance between leaf scars: Observe from the middle third from the top during active vegetative growth.

Characteristic 17: Growth habit of stem : Observe from the middle third from the top during active vegetative growth (06).

Straight (3)

Zig zag(7)

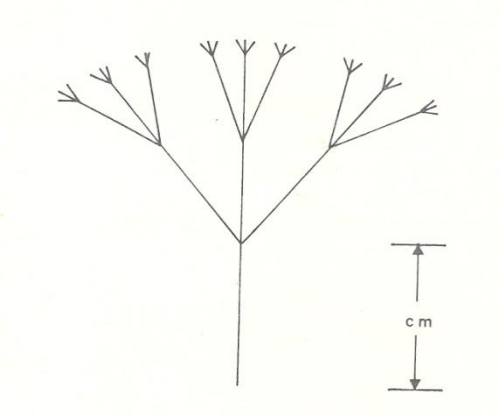
Characteristic 18. Plant type: Record the most frequent occurrence on the plant before harvest

Erect(1)

Semi- spreading(3)

Spreading(5)

Characteristic 19. Plant height (cm): Measure vertical height from the ground to the top of the canopy. Express in cm.



Characteristic 20. Plant branching habit : Record number of divisions of branching. Ignore if side branching Record the most frequent occurrence before harvest.

Single(1) Dichotomous(2) Trichotomous(3) Tetrachotomous(4)

Characteristic 21. Plant canopy: Record the most frequent occurrence on the plot during active vegetative growth.

Characteristic 23. Crop maturity (months): Record the weight of tuber, dry matter and starch content from three plants and record the average on monthly intervals from fifth month onwards. Proportion of root dry matter yield at 5-7 month compared with dry matter yield at 10^{th} month

Characteristic 24. Tuber peduncle : Record the most frequent occurrence on mature tuber

Absent(1)

Present(9)

Characteristic 25. Tuber shape : Record the most frequent occurrence on mature tubers from three plants during harvestable maturity

Conical(1)

Conico-cylindrical(2)

Cylindrical(3)

Irregular(4)

Characteristic 26 Tuber peelability: The adherence should be assessed by removing the cortex by hand from the middle third of freshly harvested root tubers.

Easy (1) = Without any breakage of cortex

Medium(5) = Minimal breakage of cortex

Difficult(7) = A lot of breakage of cortex

Characteristics 27. Tuber skin colour: Record the most frequent occurrence on tubers at harvestable maturity .

Characteristic 28. Tuber rind colour: Record the most frequent occurrence on tubers at harvestable maturity . To be recorded immediately after the tuber being cut open

Characteristic 29. Tuber flesh colour : Record the most frequent occurrence on tubers at harvestable maturity .

Characteristic 30. Mealiness of cooked tuber: Healthy cassava tubers to be selected from each variety, peeled and cut with a kitchen knife into roughly uniform-sized slices of approximately 30 g. Only the mid-portions of the tubers may be used to avoid variation caused by differential flesh coloration. Ten root pieces of about 7 cm length and 6 cm diameter may be immersed in vessels in boiling water (500 ml) on a gas stove and left to cook during 30 min. Mealiness was determined from 10 average sized boiled roots selected at random, one from each block. Mealiness was judged with fingers by experienced panelists using a scale of 1-3, where: 1= fairly mealy, 2= moderately mealy, 3= Very mealy (Ngeve, 2003)

IX. Working Group Details

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X. DUS testing Centre(s) :

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