

EAPVPF

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EAST ASIA PLANT VARIETY PROTECTION FORUM

Cassava

Manihot esculenta (Crantz.)

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

Alternative Names:

<i>Botanical name</i>	<i>English</i>	<i>Thai</i>		
<i>Manihot esculenta</i> (Crantz.)	Cassava	Man sampalang		

The purpose of these guidelines (“Test Guidelines”) is to fulfill the activities under the EAPVP Forum on harmonization of Test Guidelines.

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Manihot esculenta* Crantz.

In the case of ornamental varieties, it may, in particular, be necessary to use additional characteristics to those included in the Table of Characteristics in order to examine Distinctness, Uniformity and Stability.

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of cuttings.

2.3 The minimum quantity of plant material, to be supplied by the applicant, should be:
At least 30 cuttings, each one with a length of at least 25 cm with at least 5 buds.

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

2.5 The plant material should not have undergone any treatment which would 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.

3. Method of Examination

3.1 *Number of Growing Cycles*

The minimum duration of tests should normally be a single growing cycle. Further growing cycle can be added if necessary.

3.2 *Testing Place*

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness".

3.3 *Conditions for Conducting the Examination*

The tests 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.

3.4 *Test Design*

3.4.1 Each test should be designed to result in a total of at least 20 plants, spacing at least 1 X 1 m, which should be divided between two or more replicates.

3.4.2 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.

3.5 *Additional Tests*

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 *Distinctness*

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

4.1.2 Consistent Differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear Differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.1.4 Number of Plants / Parts of Plants to be examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 10 plants or parts taken from each of 10 plants and any other observations made on all plants in the test, disregarding any off-type plants.

4.1.5 Method of Observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the second column of the Table of Characteristics (see document TGP/9 “Examining Distinctness”, Section 4 “Observation of characteristics”):

MG: single measurement 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 observation of individual plants or parts of plants

Type of observation: visual (V) or measurement (M)

“Visual” observation (V) is an observation made on the basis of the expert’s judgment. For the purposes of this document, “visual” observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. color charts). Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

Type of record: for a group of plants (G) or for single, individual plants (S)

For the purposes of distinctness, observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded as records for a number of single, individual plants or parts of plants (S). In most cases, “G” provides a single record per variety and it is not possible or necessary to apply statistical methods in a plant-by-plant analysis for the assessment of distinctness.” In cases where more than one method of observing the characteristic is indicated in the Table of Characteristics (e.g. VG/MG), guidance on selecting an appropriate method is provided in document TGP/9, Section 4.2.

4.2 *Uniformity*

4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

4.2.2 For the assessment of uniformity of clones, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 20 plants, 1 off-type is allowed.

4.3 *Stability*

4.3.1 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability may be tested, either by growing a further generation, or by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the previous material supplied.

5. Grouping of Varieties and Organization of the Growing Trial

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

- (a) Apical leaf: color (characteristic 13)
- (b) Apical leaf: pubescence (characteristic 14)
- (c) Petiole: color (characteristic 23)
- (d) Leaf: variegation (characteristic 16)
- (e) Petiole: attitude in relation to stem (characteristic 21)
- (f) Stem: color of exterior (characteristic 3)
- (g) Stem: growth habit (characteristic 5)
- (h) Root: external color (characteristic 27)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction and document TGP/9 “Examining Distinctness”.

6. Introduction to the Table of Characteristics

6.1 *Categories of Characteristics*

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

6.2 *States of Expression and Corresponding Notes*

6.2.1 States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

6.2.2 In the case of qualitative and pseudo-qualitative characteristics (see Chapter 6.3), all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines

may be abbreviated as follows:

State	Note
small	3
medium	5
large	7

However, it should be noted that all of the following 9 states of expression exist to describe varieties and should be used as appropriate:

State	Note
very small	1
very small to small	2
small	3
small to medium	4
medium	5
medium to large	6
large	7
large to very large	8
very large	9

6.2.3 Further explanation of the presentation of states of expression and notes is provided in document TGP/7 “Development of Test Guidelines”.

6.3 *Types of Expression*

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 *Example Varieties*

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

6.5 *Legend*

(*) Asterisked characteristic – see Chapter 6.1.2

QL: Qualitative characteristic – see Chapter 6.3

QN: Quantitative characteristic – see Chapter 6.3

PQ: Pseudo-qualitative characteristic – see Chapter 6.3

MG, MS, VG, VS – see Chapter 4.1.5

(a)-(b) See Explanations on the Table of Characteristics in Chapter 8.1

(+) See Explanations on the Table of Characteristics in Chapter 8.2

7. Table of Characteristics

			Example variety	Note
1	MS	Plant: height		
(*)				
QN	(b)	short		3
		medium		5
		tall		7
2	VG	Stem: color of cortex		
PQ	(b)	light green		1
		dark green		2
		cream		3
		purplish	RED cassava	4
3	VG	Stem: color of exterior		
(*)				
PQ	(b)	orange		1
		greyish yellow		2
		greenish yellow		3
		brownish yellow		4
		light brown		5
		dark brown		6
		grey		7
4	VG	Stem: color of epidermis (middle part of plant)		
PQ	(b)	cream		1
		light brown		2
		dark brown		3
		orange		4
		purple		5

			Example variety	Note
5 (* (+)	VG	Stem: growth habit		
QL	(b)	straight		1
		zigzag		2
6	VG	Stem: prominence of leaf scars (middle part of plant)		
QN	(b)	weak		3
		medium		5
		strong		7
7 (+)	VG	Young stem: main color		
PQ	(a)	light green	MM122	1
		yellowish green	MM183	2
		green	MM181	3
8	VG	Young stem: presence of anthocyanin coloration		
QL	(a)	absent		1
		present		9
9	VG	Young stem: Intensity of anthocyanin coloration		
QN	(a)	weak		3
		medium		5
		strong		7
10	VG	Shoot tip: intensity of anthocyanin coloration		
QN	(a)	weak		3
		medium		5
		strong		7

			Example variety	Note
11	MG	Stem: number of branching level		
(+)				
QN	(b)	absent		1
		one		2
		two		3
		three		4
		more than three		5
12	MS	Stem: distance between leaf scars on nodes		
(+)				
QN	(b)	short		3
		medium		5
		long		7
13	VG	Apical leaf: color		
(*)				
(+)				
PQ	(a)	light green	Rayong 3	1
		dark green	Rayong 90	2
		purplish green		3
		purple	Rayong 1	4
		light brown	Hauybong 60	5
14	VG	Apical leaf: pubescence		
(*)				
(+)				
QL	(a)	absent	Rayong 9	1
		present	Rayong 1	9
15	VG	Leaf: color		
PQ	(a)	light green		1
		dark green		2
		purplish green		3
		purple		4

			Example variety	Note
16	VG	Leaf: variegation		
(*)				
QL	(a)	absent		1
		present		9
17	MS	Leaf: length of central lobe		
(*)				
(+)				
QN	(a)	short		3
		medium		5
		long		7
18	MS	Leaf: width of central lobe		
(*)				
(+)				
QN	(a)	narrow		3
		medium		5
		broad		7
19	VG	Leaf: predominant shape of central lobe		
(*)				
(+)				
PQ	(a)	linear		1
		obovate		2
		oblong		3
20	VG	Leaf: color of veins		
(+)				
PQ	(a)	green	Rayong 90	1
		reddish green	Rayong 5	2
		red		3
		purple		4
21	VG	Petiole: attitude in relation to stem		
(*)				
(+)				
PQ	(a)	semi erect		1
		horizontal		2
		drooping		3
		irregular		4

			Example variety	Note
22	MS	Petiole: length		
(*)				
QN	(a)	short		3
		medium		5
		long		7
23	VG	Petiole: color		
(*)				
(+)				
PQ	(a)	yellowish green		1
		light green	Rayong 90	2
		green		3
		reddish green		4
		greenish red		5
		red		6
		purple		7
24	VG	Stipule: length		
(*)				
(+)				
QN	(a)	short		3
		medium		5
		long		7
25	VG	Stipule: margin		
(*)				
(+)				
QL	(a)	entire		1
		split		2
26	VG	Root : extent of neck		
(*)				
(+)				
QL	(b)	absent		1
		present		9

			Example variety	Note
27	VG	Root: external color		
(+)				
PQ	(b)	whitish		1
		light brown		2
		medium brown		3
		dark brown		4
		reddish brown	MM122	5
28	VG	Root: color of cortex		
PQ	(b)	white		1
		cream		2
		yellow		3
		pink		4
		light brown		5
		purple		6
29	VG	Root: color of flesh		
PQ	(b)	white		1
		cream		2
		yellow		3
		pink		4
30	VG	Root: texture of epidermis		
QL	(b)	smooth		1
		rough		2
31	VG	Root: shape		
(+)				
PQ	(b)	conical		1
		conical to cylindrical		2
		cylindrical		3
		irregular		4

			Example variety	Note
32	MG	Root: cyanide content		
(+)				
QN	(b)	low		3
		medium		5
		high		7
33	VG	Root: adherence of cortex to flesh		
(+)				
QN	(b)	weak		3
		medium		5
		strong		7
34	MG	Root: content of starch in flesh		
(+)				
QN	(b)	low		3
		medium		5
		high		7

8. Explanations on the Table of Characteristics

8.1 *Explanations covering several characteristics*

Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

(a) Observations should be made after 90-180 days (3-6 months) from planting as leaf characteristics at the middle part of canopy as fully developed leaf

(b) Observations should be made after 240 -360 days (8-12 months) from planting

8.2 *Explanations for individual characteristics*

Ad. 5: Stem: growth habit



1
straight



2
zigzag

Ad. 7: Young stem: main color



1
light green

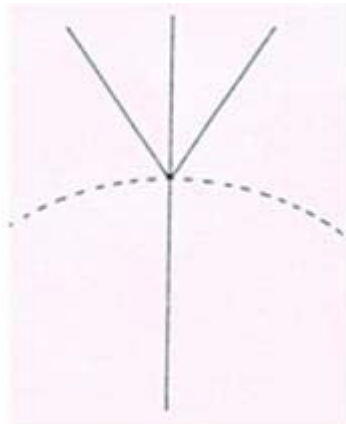


2
yellowish green

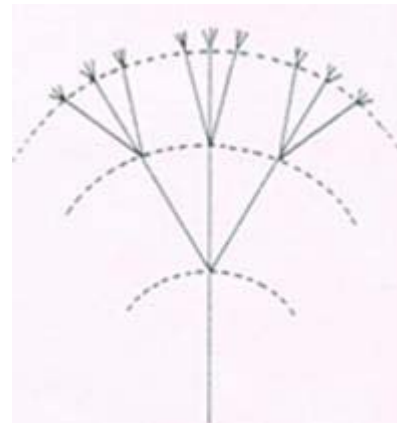


3
green

Ad. 11: Stem: number of branching level



2
one branching



4
three branching

Ad. 12: Stem: distance between leaf scars on nodes

The characteristic should be observed at the middle part of the plant, and two scars in the same alignment are to be observed.

Ad. 13: Apical leaf: color



1
light green

2
dark green

3
purplish green

4
purple

5
light brown

Ad. 14: Apical leaf: pubescence



1
absent

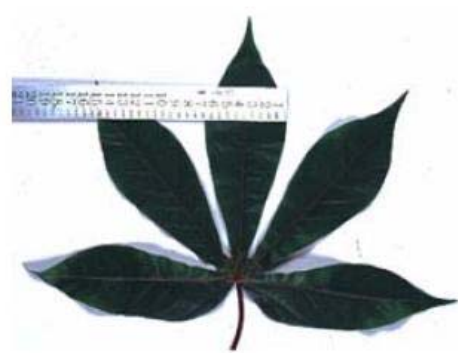


9
present

Ad. 17: Leaf: length of central lobe



Ad. 18: Leaf: width of central lobe



Ad. 19: Leaf: predominant shape of central lobe



1
linear



2
obovate



3
oblong

Ad. 20: Leaf: color of veins



1
green



2
reddish green



3
red

Ad. 21: Petiole: attitude in relation to stem



1
semi erect



2
horizontal



3
drooping



4
irregular

Ad. 23: Petiole: color



1
yellowish green



2
light green



3
green



4
reddish green



5
greenish red



6
red



7
purple

Ad. 24: Stipule: length



3
short



5
medium



7
long

Ad. 25: Stipule: margin

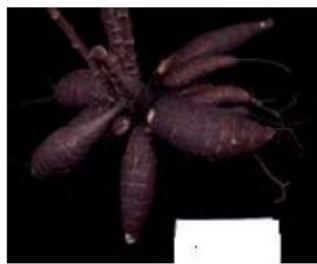


1
entire



2
split

Ad. 26: Root: extent of neck



1
absent



9
present

Ad. 27: Root: external color



5
reddish brown

Ad. 31: Root: shape



1
conical



2
conical to cylindrical



3
cylindrical



4
irregular

Ad. 32: Root: cyanide content

Rapid screening assay of cyanide content of cassava (Williams and Edward (1980) method) This is a rapid, inexpensive screening assay developed in order to measure the cyanide content of cassava (*Manihot esculenta* Crantz.) tubers. A small disc of parenchyma tissue cut with a cork borer or alternatively grated tissue placed in a stoppered glass tube with a filter paper previously spotted with a drop of tetra-base [4,4'-methylenebis-(N,N- dimethylaniline)] and cupric acetate and hydrogen. Cyanide liberated produces a blue color on the filter paper. The intensity of the blue color developed within one hour is rated visually on a graded scale from 0 to 5. The correlation coefficient between samples accurately analyzed for total cyanide and also tested using the rapid assay is 0.77.

Low cyanide content	0 to 1.9
Medium cyanide content	2.0 to 3.9
High cyanide content	4.0 to 5.0

Ad. 33 Root: adherence of cortex to flesh

Involves hand removal of root cortex from the middle third of freshly harvested root tuber. Weak adherence is when the cortex is removed round the root tuber without any breakage while strong adherence is when peeling of the cortex exhibits a lot of breaking and for medium adherence there is minimal breaking of the cortex.

Ad. 34: Root: content of starch in flesh

Heritability for DM in cassava is relatively high; 0.87 broad sense heritability and 0.51 – 0.67 narrow sense heritability (Kawano *et al.* 1987). Estimation of DM and starch content in cassava is based on the principle of a linear relationship between specific gravity with DM and or starch content. Percentage DM = $158.3x - 142$, while starch content = $112.1x - 106.4$; where x = specific gravity. Specific gravity is measured according to the following methodology:

- 1) Prepare root samples weighing 3–5 kg.
- 2) Weigh sample in air (W_a) using a suitable balance. Ensure that that the roots are generally free from soil and other debris
- 3) Weigh the sample in water (W_w).
- 4) Ensure that you use the same container to weigh both in air and in water. A sturdy wire basket works perfectly as it allows soil debris to fall through and also allows easy measurement in water.
- 5) Compute specific gravity at $W_w/(W_a - W_w)$
- 6) Compute DM and starch content using the formulas
 $DM = 158.3x - 142$, and starch content = $112.1x - 106.4$.

9. Literature

Fukuda, W.M.G., C.L. Guevara, R. Kawuki, and M.E. Ferguson. 2010. Selected morphological and agronomic descriptors for the characterization of cassava. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 19 pp.

10. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
		Application date: (not to be filled in by the applicant)
TECHNICAL QUESTIONNAIRE to be completed in connection with an application for plant breeders' rights		
1. Subject of the Technical Questionnaire		
1.1 Botanical Name	<input type="text" value="Manihot esculenta Crantz."/>	
1.2 Common Name	<input type="text" value="Cassava"/>	
2. Applicant		
Name	<input type="text"/>	
Address	<input type="text"/>	
Telephone No.	<input type="text"/>	
Fax No.	<input type="text"/>	
E-mail address	<input type="text"/>	
Breeder (if different from applicant)	<input type="text"/>	
3. Proposed denomination and breeder's reference		
Proposed denomination (if available)	<input type="text"/>	
Breeder's reference	<input type="text"/>	

4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross
(please state parent varieties)

(.....) X (.....)
female parent male parent

(b) partially know cross
(please state know parent variety (ies))

(.....) X (.....)
female parent male parent

(c) unknow cross

4.1.2 Mutation
(please state parent variety)

4.1.3 Discovery and development
(please state where and when discovered and how developed)

4.1.4 Other
(please provide details)

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
<p>4.2 Method of propagating the variety</p> <p>4.2.1 Vegetative propagation</p> <p>(a) cuttings []</p> <p>(b) <i>in vitro</i> propagation []</p> <p>(c) other (state method) []</p> <div data-bbox="347 712 1300 790" style="border: 1px solid black; height: 35px; margin: 10px 0;"></div> <p>4.2.2 Seed []</p> <p>4.2.3 Other [] (please provide details)</p> <div data-bbox="347 1048 1300 1126" style="border: 1px solid black; height: 35px; margin: 10px 0;"></div>		

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).

Characteristics	Example Varieties	Note
5.1 Apical leaf: color (13)		
light green	Rayong 3	1[]
dark green	Rayong 90	2[]
purplish green		3[]
purple	Rayong 1	4[]
light brown	Hauybong 60	5[]
5.2 Apical leaf: pubescence (14)		
absent	Rayong 9	1[]
present	Rayong 1	9[]
5.3 Leaf: predominant shape of central lobe (19)		
linear		1[]
obovate		2[]
oblong		3[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.4 Petiole: color (23)		
yellowish green		1[]
light green	Rayong 90	2[]
green		3[]
reddish green		4[]
greenish red		5[]
red		6[]
purple		7[]
5.5 Leaf: variegation (16)		
absent		1[]
present		9[]
5.6 Petiole: attitude in relation to stem (21)		
semi erect		1[]
horizontal		2[]
drooping		3[]
irregular		4[]
5.7 Stipule: length (24)		
short		3[]
medium		5[]
long		7[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.8 Stem: color of exterior (3)		
orange		1[]
greyish yellow		2[]
greenish yellow		3[]
brownish yellow		4[]
light brown		5[]
dark brown		6[]
grey		7[]
5.9 Stem: growth habit (5)		
straight		1[]
zigzag		2[]
5.10 Root: external color (27)		
whitish		1[]
light brown		2[]
medium brown		3[]
dark brown		4[]
reddish brown	MM122	5[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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6. Similar varieties and differences from these varieties

Please use the follow table and box for comments, to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the similar variety(ies)	Describe the expression of the characteristic(s) for your candidate variety

Comments:

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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7. Additional information which may help in the examination of the variety

7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

Yes [] No []

(If yes, please provide details)

7.2 Special conditions for the examination of the variety

Are there any special conditions for growing the variety or conducting the examination?

Yes [] No []

(If yes, please give details)

7.3 Other information

8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [] No []

(b) Has such authorization been obtained?

Yes [] No []

If the answer to (b) is yes, please attach a copy of the authorization.

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9. Information on plant material to be examined or submitted for examination.

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

- | | | |
|---|---------|--------|
| (a) Microorganisms (e.g. virus, bacteria, phytoplasma) | Yes [] | No [] |
| (b) Chemical treatment (e.g. growth retardant or pesticide) | Yes [] | No [] |
| (c) Tissue culture | Yes [] | No [] |
| (d) Other factors | Yes [] | No [] |

Please provide details for where you have indicated "yes".

.....

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant's name

Signature

Date

[End of document]