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CIP Research Guide 36

MORPHOLOGIC IDENTIFICATION OF DUPLICATES IN COLLECTIONS OF Ipomoea batatas

1992

Zosimo Huaman

CIP T3 H83



INTERNATIONAL POTATO CENTER (CIP)
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Huaman, Z. 1992. Morphologic identification of duplicates in collections of *Ipomoea batatas*. CIP Research Guide 36. International Potato Center, Lima, Peru. 28 pp.

CIP Research Guide 36

MORPHOLOGIC IDENTIFICATION OF DUPLICATES IN COLLECTIONS OF *Ipomoea batatas*

- 1 Introduction
- 2 Morphologic evaluation
- 3 Data processing
- 4 Duplicate verification

1 INTRODUCTION

Duplicates in sweet potato collections are samples of the same cultivar that were collected in different farms of one or more localities. Their presence increases the cost of maintenance of these collections and slow down the process of evaluation for desirable traits for use in breeding.

In many countries, there are several collections maintained by universities, experiment stations or private farmers. When a national sweet potato collection is organized, it is recommended:

- 1. To introduce all those collections in one site to grow them under the same environment.
- 2. To record some of their key morphologic characters to facilitate their grouping according to similarities in their vine, leaf and storage root characters.
- 3. To organize a new planting of the collection according to these groupings, and to make more detailed morphological comparisons between those accessions that are morphologically alike.

The identification of duplicates in sweet potato collections depends on the degree of accuracy with which the data were obtained during the morphological characterization in the field; on the correct transfer of the data to a file for computer processing; and on a good validation of the data once the accessions of the collection are grouped according to their similarities.

2 MORPHOLOGIC EVALUATION

An adequate morphologic characterization of a sweet potato collection is achieved when the data are recorded on plants from the same planting date, growing under the same environment, at the same plant density, and in the most favorable season for good plant development.

The time to record the data is when the plants are in a stage of good growth. Under normal conditions, it is recommended to record the morphologic data at about 90 days from planting or 10 days before harvest in early maturing cultivars.

Descriptor states related to length or size are scored on the basis of the average value of measurements made on several plants of each accession.

Considering that many sweet potato characters of the vines and leaves vary throughout the plant, it is necessary to choose a part of the plant where they are mature. In the apical part of the plant, leaves are very young, whereas in the basal part, they are too old. Therefore, it is recommended to record these characters in a section located in the middle portion of the main stem. The data to be recorded will be the average expression of at least 3 leaves, 3 internodes, etc. located in this section.

Since many times the number of accessions in the collection is increased with new donations or collections, the morphologic data for these new material should be recorded in a similar season to that used to describe the main collection. Moreover, it is recommended to include in the same field some cultivars of reference representing each plant type, stem thickness, leaf shape, etc. This will allow to make comparisons between the data recorded in different years.

LIST OF DESCRIPTORS CONSIDERED AS KEY CHARACTERS FOR DUPLICATE IDENTIFICATION

1. Vine Characters

1.1 Twining

Ability of vines to climb stems or plants.

- 0 Non-twining
- 3 Slightly twining
- 5 Moderately twining
- 7 Twining
- 9 Very twining

1.2 Plant type

Determined by the length of the main vines.

- 3 Erect (< 75 cm)
- 5 Semi-compact (75 150 cm)
- 7 Spreading (151 250 cm)
- 9 Extremely spreading (> 250 cm)

1.3 Vine internode diameter and length

Average expression of at least three internodes located in the middle section of the vine.

1.3.1 Internode diameter

- 1 Very thin (< 4 mm)
- 3 Thin (4-6 mm)
- 5 Intermediate (7-9 mm)
- 7 Thick (10-12 mm)
- 9 Very thick (>12 mm)

1.3.2 Internode length

- 1 Very short (<3 cm)
- 3 Short (3-5 cm)
- 5 Intermediate (6-9 cm)
- 7 Long (10-12 cm)
- 9 Very long (> 12 cm)

1.4 Vine pigmentation

Anthocyanin (purple) pigmentation present in the vines besides the green color. The predominant color should be evaluated considering the whole vine from base to tip. The secondary color is more easily evaluated using younger vines.

1.4.1 Predominant color of vine

- 1 Green
- 3 Green with few purple spots
- 4 Green with many purple spots
- 5 Green with many dark purple spots
- 6 Mostly purple
- 7 Mostly dark purple
- 8 Totally purple
- 9 Totally dark purple

1.4.2 Secondary color of vine

- 0 Absent
- 1 Green base
- 2 Green tip
- 3 Green nodes
- 4 Purple base
- 5 Purple tip
- 6 Purple nodes
- 7 Other

1.5 Vine tip pubescence

Degree of hairiness of immature leaves recorded from the apex of the vines.

- 0 None
- 3 Sparse
- 5 Moderate
- 7 Heavy
- 9 Very heavy

2. Leaf Characters

2.1 Mature leaf shape

Described by the most common expression of the outline of the leaf (Fig. 1), type of leaf lobes (Fig. 2); the average total number of lobes (Fig. 3); and the shape of the central lobe (Fig. 4) of leaves located in the middle section of the vine of 90 days old plants.

2.1.1 General leaf outline

- 1 Rounded
- 2 Reniform (kidney-shaped)
- 3 Cordate (heart-shaped)
- 4 Triangular
- 5 Hastate (Trilobular, spear-shaped, with the basal lobes more or less divergent)
- 6 Lobed
- 7 Almost divided

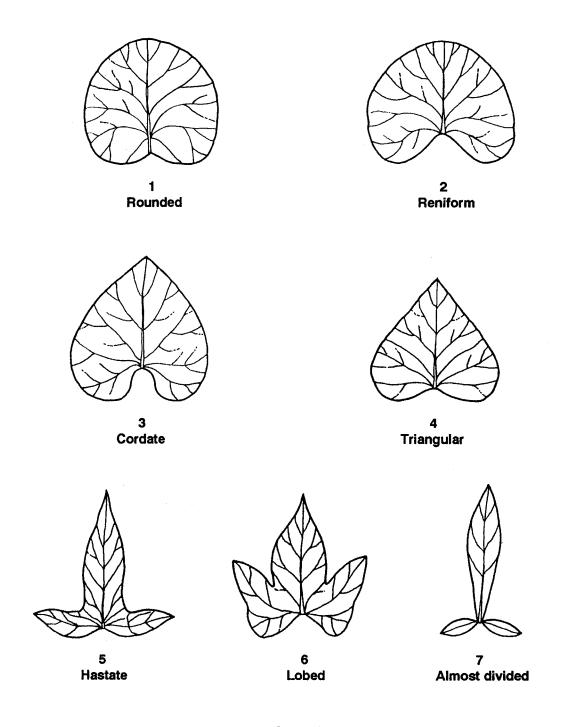


Figure 1. General leaf outline.

2.1.2 Type of leaf lobes

- 0 No lateral lobes (entire)
- 1 Very slight (teeth)
- 3 Slight
- 5 Moderate
- 7 Deep
- 9 Very deep

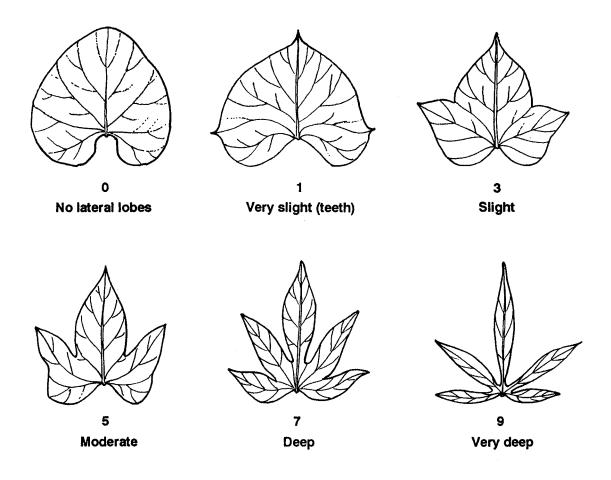


Figure 2. Type of leaf lobes.

2.1.3 Number of leaf lobes

Most leaves of sweet potatoes have two basal lobes and they should not be counted. Therefore, the data to be recorded is the predominant number of lateral and central leaf lobes observed in the leaves located in the middle section of the vine in 90 days old plants.

Generally sweet potatoes have 1, 3, 5, 7 or 9 leaf lobes. If the leaf has no lateral lobes but shows a central teeth this number is 1. If the apical portion of the leaf is totally rounded this number is 0. Record the number of lobes as 9 when this number is 9 or more, as it is the case of some leaves with more than 9 teeth on the leaves.

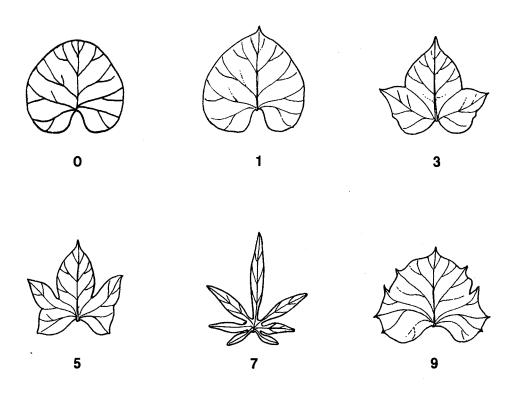


Figure 3. Number of leaf lobes.

2.1.4 Shape of central leaf lobe

- 0 Absent
- 1 Teeth
- 2 Triangular
- 3 Semi-circular
- 4 Semi-elliptic
- 5 Elliptic
- 6 Lanceolate
- 7 Oblanceolate
- 8 Linear (broad)
- 9 Linear (narrow)

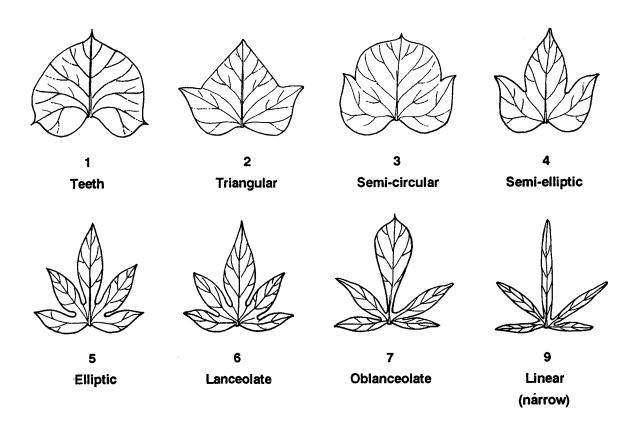


Figure 4. Shape of central leaf lobe.

2.2 Mature leaf size

Length from the basal lobes to the tip of the leaves. Record the average expression of at least 3 leaves located in the middle section of the vine (Fig. 5).

- 3 Small (< 8 cm)
- 5 Medium (8 15 cm)
- 7 Large (16 25 cm)
- 9 Very large (> 25 cm)

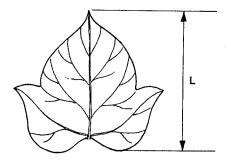


Figure 5. Mature leaf size.

2.3 Abaxial leaf vein pigmentation

Describe the most frequent expression of the distribution of anthocyanin (purple) pigmentation shown in the veins of the lower surface of leaves (Fig. 6).

- 1 Yellow
- 2 Green
- 3 Purple spot at base of main rib
- 4 Purple spots in several veins
- 5 Main rib partially purple
- 6 Main rib mostly or totally purple
- 7 All veins partially purple
- 8 All veins mostly or totally purple
- 9 Lower surface and veins totally purple

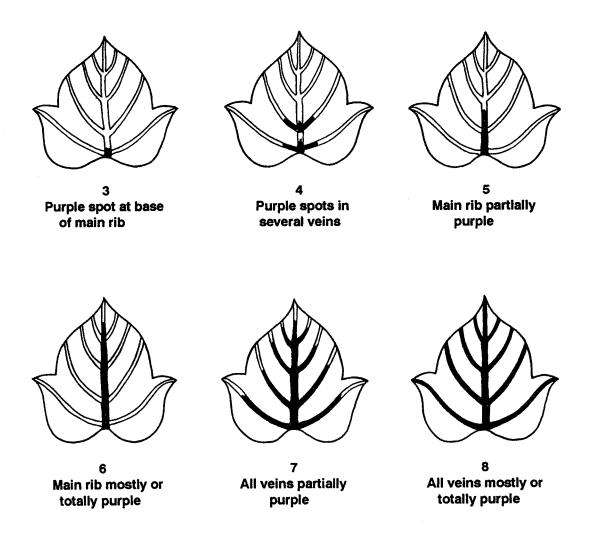


Figure 6. Abaxial leaf vein pigmentation.

2.4 Foliage color

Describe the overall foliage color considering the color of fully expanded mature and immature leaves shown by several plants. The variegation in leaf color due to virus symptoms should not be recorded.

2.4.1 Mature leaf color

- 1 Yellow-green
- 2 Green
- 3 Green with purple edge
- 4 Greyish (due to heavy pubescence)
- 5 Green with purple veins on upper surface
- 6 Slightly purple
- 7 Mostly purple
- 8 Green upper, purple lower
- 9 Purple both surfaces

2.4.2 Immature leaf color

- 1 Yellow-green
- 2 Green
- 3 Green with purple edge
- 4 Greyish (due to heavy pubescence)
- 5 Green with purple veins on upper surface
- 6 Slightly purple
- 7 Mostly purple
- 8 Green upper, purple lower
- 9 Purple both surfaces

2.5 Petiole pigmentation and length

2.5.1 Petiole pigmentation

Distribution of anthocyanin (purple) pigmentation in the petioles of leaves. Indicate the most predominant color first.

- 1 Green
- 2 Green with purple near stem
- 3 Green with purple near leaf
- 4 Green with purple at both ends
- 5 Green with purple spots throughout petiole
- 6 Green with purple stripes
- 7 Purple with green near leaf
- 8 Some petioles purple, others green
- 9 Totally or mostly purple

2.5.2 Petiole length

Average petiole length, from the base to the insertion with the blade, of at least 3 leaves in the middle portion of a main vine (Fig. 7).

- 1 Very short (< 10 cm)
- 3 Short (10 20 cm)
- 5 Intermediate (21 30 cm)
- 7 Long (31 40 cm)
- 9 Very long (> 40 cm)

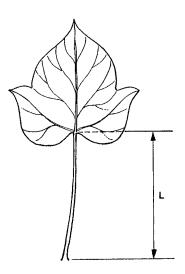


Figure 7. Petiole length.

3. Storage Root Characters

Record all storage root descriptors considering the most representative expression of the character shown in medium- to large-sized storage roots of several plants.

3.1 Storage root shape

Describe the storage root outline shown in a longitudinal section (Fig. 8).

- 1 Round almost a circular outline with a length to breadth (L/B) ratio of about 1 to 1.
- 2 Round elliptic a slightly circular outline with acute ends. L/B ratio not more than 2 to 1.
- 3 Elliptic outline with about the maximum breadth at equal distance from both ends which are slightly acute. L/B ratio not more than 3 to 1.
- 4 Obovate outline resembling the longitudinal section of an egg. The broadest part is at the distal end (i.e. opposite to the root stock).
- 5 Ovate inversely ovate outline. The broadest part is at the proximal end (i.e. close to the root stalk).
- 6 Oblong almost rectangular outline with sides nearly parallel and corners rounded. L/B ratio about 2 to 1.
- 7 Long oblong oblong outline with a L/B ratio of more than 3 to 1.
- 8 Long elliptic elliptic outline with a L/B ratio of more than 3 to 1.
- 9 Long irregular or curved.

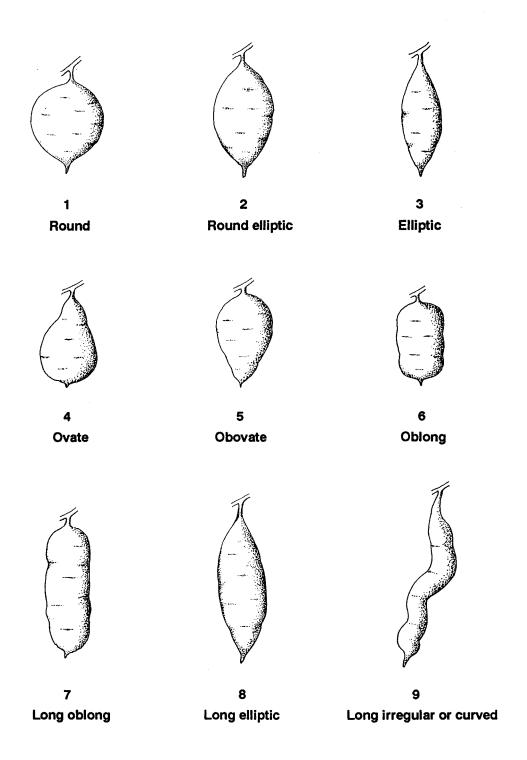


Figure 8. Storage root shape.

3.2 Storage root defects

- 0 Absent
- 1 Alligator's like skin
- 2 Veins
- 3 Shallow horizontal constrictions
- 4 Deep horizontal constrictions
- 5 Shallow longitudinal grooves
- 6 Deep longitudinal grooves
- 7 Deep constrictions and deep grooves
- 8 Other

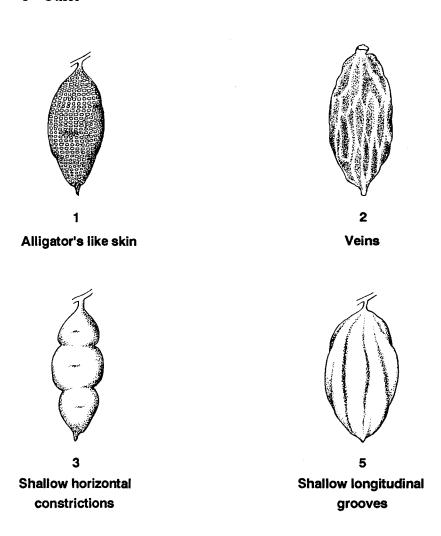


Figure 9. Storage root defects.

3.3 Storage root cortex thickness

- 1 Very thin (1 mm or less)
- 3 Thin (1-2 mm)
- 5 Intermediate (2-3 mm)
- 7 Thick (3-4 mm)
- 9 Very thick (more than 4 mm)

3.4 Storage root skin color

Many freshly harvested storage roots should be washed and cured prior to evaluation. Describe the predominant color, its intensity, and the secondary color. The most representative skin color observed in the cultivar should be recorded.

3.4.1 Predominant skin color

- 1 White
- 2 Cream
- 3 Yellow
- 4 Orange
- 5 Brownish orange
- 6 Pink
- 7 Red
- 8 Purple-red
- 9 Dark purple

3.4.2 Intensity of predominant skin color

- 1 Pale
- 2 Intermediate
- 3 Dark

3.4.3 Secondary skin color

- 0 Absent
- 1 White
- 2 Cream
- 3 Yellow
- 4 Orange
- 5 Brownish orange
- 6 Pink
- 7 Red
- 8 Purple-red
- 9 Dark purple

3.5 Storage root flesh color

Describe the predominant color, secondary color and the distribution of the secondary color, from cross and longitudinal sections made about the middle of freshly harvested storage roots.

3.5.1 Predominant flesh color

- 1 White
- 2 Cream
- 3 Dark cream
- 4 Pale yellow
- 5 Dark yellow
- 6 Pale orange
- 7 Intermediate orange
- 8 Dark orange
- 9 Strongly pigmented with anthocyanins

3.5.2 Secondary flesh color

- 0 Absent
- 1 White
- 2 Cream
- 3 Yellow
- 4 Orange
- 5 Pink
- 6 Red
- 7 Purple-red
- 8 Purple
- 9 Dark purple

3.5.3 Distribution of secondary flesh color

- 0 Absent
- 1 Narrow ring in cortex
- 2 Broad ring in cortex
- 3 Scattered spots
- 4 Narrow ring in flesh
- 5 Broad ring in flesh
- 6 Ring and other areas in flesh
- 7 In longitudinal sections
- 8 Covering most of the flesh
- 9 Covering all flesh

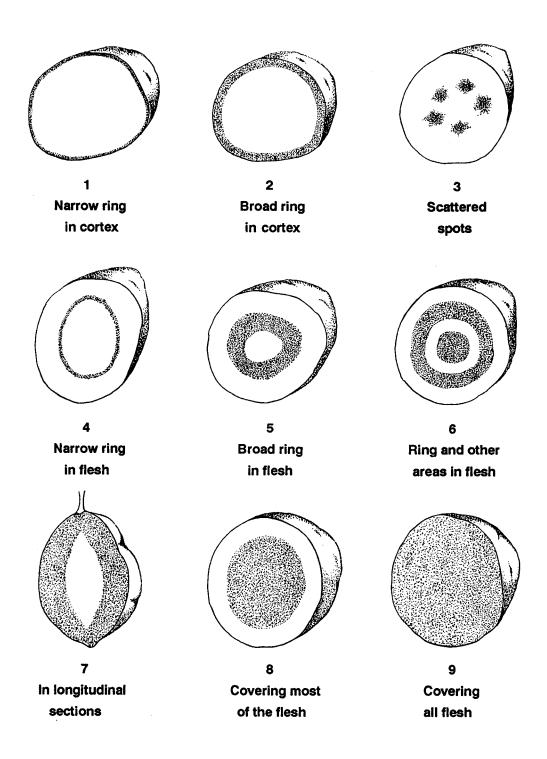


Figure 10. Distribution of secondary flesh color.

3.6 Storage root arrangement

Description of the arrangement of the storage roots on the underground stems (Fig. 11).

- 1 Closed cluster
- 3 Open cluster
- 5 Disperse
- 7 Very disperse

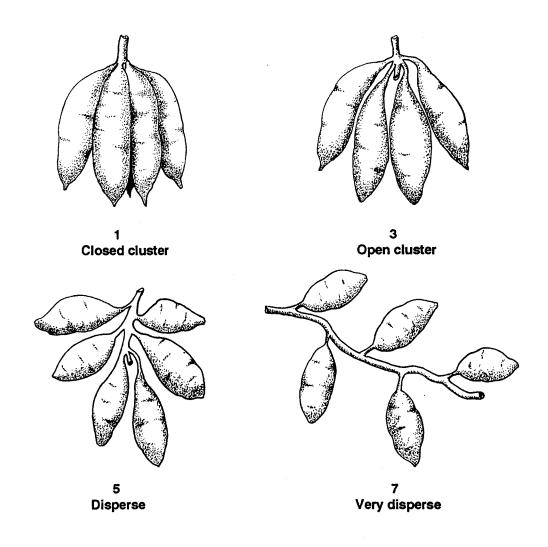


Figure 11. Storage root arrangement.

3 DATA PROCESSING

The morphological data of accessions of a sweet potato collection can be recorded on forms showing all descriptors in columns (Table 1). It is also convenient to have a table with all the descriptor codes to secure a correct data recording (Table 2). Many times is needed to look at the figures of those descriptors that show shape variation until one is used to all the different options.

Once the data recording has been finished in the field, they have to be organized in a microcomputer database using commercial programs (DBASE, SYMPHONY, etc.) to allow an easy data handling. It is a good practice to proof read the original data with a printout of the database as soon as the data entering has been completed.

An easy way to group the accessions of the collection according to their similarities is by means of a sorting based on those descriptors that are least affected by the environment. This sorting can be made on the basis of the following descriptors: mature leaf shape, abaxial leaf vein pigmentation, petiole pigmentation, vine internode diameter, vine pigmentation, and storage root skin and flesh colors. This will put together all accessions with the same value for leaf shape, which will then be sorted on the code for abaxial leaf vein pigmentation, and so on for the other descriptors. Moreover, all accessions with the same data for those descriptors will be printed together.

There are more sophisticated programs for this type of analyses such as CLUSTER ANALYSIS in S.A.S., in which not only all the groups with equal data are listed together; but also those with differences in 1, 2, etc. descriptors. The advantage of this method over the simple sorting is that you can get a list of accessions with identical data. The disadvantage is that if the number of accessions is very large, the analyses has to be made in small groups.

If the collection is planted on the basis of these groupings, obtained either by the sorting of the most important characters or by cluster analyses, it is easier to verify consistency of the data. It is not unusual that the same cultivar can show the different data in some characters. This can be due to environmental effects such as the watering, fertilization, shading, etc.

4 DUPLICATE VERIFICATION

Once that the database is free of mistakes due to the subjectivity of the evaluators, the groupings of accessions with identical morphological characteristics will show the duplicates of the same sweet potato cultivar.

Since sweet potatoes show a high frequency of somatic mutations, it is not unusual to find accessions of the collection that are identical in all characteristics of plants, leaves, flowers, but they differ in the storage root skin color. Another characteristic that mutates very easely is the storage root flesh color.

Those accessions of the collection that are considered duplicates of the same cultivar must have all morphological characteristics identical. However, this duplicates must be verified in other physiological, biochemical, etc. characteristics to determine, as much as possible, if they are the same genotype.

Among other criteria to determine if two or more accessions with identical morphology are duplicates of the same cultivar, are the comparisons on flowering intensity and crossability tests. All duplicate accessions must flower more or less in the same season and with the same intensity. If they do not flower, the requierements for flower induction must be the same for all of them. In the crossability tests, it is expected that two duplicates will show the same result as when they are selfed or crossed with cultivars with known incompatibility groups.

Another test on duplicates could be to determine the reaction of their storage roots to the inoculation of pathogens. If they are the same genotype, it is expected a similar reaction to the pathogen for all duplicate accessions. In these cases, it is expected some variation between duplicate accessions similar to that obtained using many roots from one single accession.

Whenever the necessary equipment is available, a fast method to verify the results of the morphologic evaluations is by means of electrophoretic comparisons of proteins and esterases extracted from storage roots. Those accessions of the collection that are morphologically identical and have the same patterns of proteins and esterases are considered duplicates of the same cultivar.

Once the duplicates have been identified, it is advisable to transfer to *in vitro* culture at least one accession representing to each group. These accessions have also to be maintained in larger plots in the field, in order to avoid losses and to continue with other evaluations for their utilization. Furthermore, in as much as possible true seeds should be obtained from each duplicate accession before discarding them from clonal propagation.

Processed by the Communication Unit, CIP, Lima, Peru, June 1992 Copies printed: 600

TABLE 1. DATA SHEET FOR CHARACTERIZATION OF NATIONAL COLLECTIONS OF Ipomoea batatas

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	ABBA	GEMENT				<u> </u>															
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	FLESH COLOR	SECOND.																			
	E	PREDON																			
£001	-	SECOND.																			
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MATURE LEAF	SHAPE	NUMBER OF LOBES																			
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		GRAL																			
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	IDENTIFICATION NUMBER																	1.			
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TABLE 2. LIST OF KEY CHARACTERS FOR DUPLICATE IDENTIFICATION IN Ipomoea batatas COLLECTIONS.

ENTATION SECONDARY COLOR	0 Absent 1 Green base 2 Green tip 3 Green nodes 4 Purple base 5 Purple tip 6 Purple nodes 7 Other	ABAXIAL LEAF VEIN PIGMENTATION	1 Yellow 2 Green 3 Purple spot at base of main rib 4 Purple spots in several veins 5 Main rib partially purple 6 Main rib mostly or totally purple 7 All veins partially purple 8 All veins mostly or totally purple 9 Lower surface and veins totally purple	PETIOLE LENGTH	1 Very short (less than 10 cm) 3 Short (10 - 20 cm) 5 Intermediate (21 - 30 cm) 7 Long (31 - 40 cm) 9 Very long (more than 40 cm)
VINE PIGMENTATION PREDOMINANT COLOR SECON	1 Green 3 Grenn few purple spots 4 Green many purple spots 5 Green many dark purple spots 6 Mostly purple 7 Mostly dark purple 8 Totally purple 9 Totally dark purple	Ä	1 Yellow 2 Green 3 Purple s 4 Purple s 5 Main rib 6 Main rib 7 All veins 8 All veins	~	
PREDOMII		SHAPE OF CENTRAL LOBE	0 Absent 1 Teeth 2 Triangular 3 Semi-circular 4 Semi-elliptic 5 Elliptic 6 Lanceolate 7 Oblanceolate 8 Linear (broad) 9 Linear (narrow)	PETIOLE PIGMENTATION	1 Green 2 Green with purple near stem 3 Green with purple near leaf 4 Green with purple at both ends 5 Green with purple spots throughout petiole 6 Green with green near leaf 7 Purple with green near leaf 8 Some petioles purple, others green 9 Totally or mostly purple
E LENGTH	1 Very short (< 3 cm) 3 Short (3 - 5 cm) 5 Intermediate (6 - 9 cm) 7 Long (10 - 12 cm) 9 Very long (> 12 cm)	۳. پن	0 - 0 6 4 6 6 7 8 6	PETIOL	1 Green 2 Green with purple near stem 3 Green with purple near leaf 4 Green with purple at both en 5 Green with purple spots throug 6 Green with purple stripes 7 Purple with green near leaf 8 Some petioles purple, others 9 Totally or mostly purple
VINE INTERNODE		LEAF SHAPE NUMBERC LEAFLOBE	uding the two bas	LOR INMATURE LEAF COLOR	ple edge 7 pubescence) ple veins ce ce
DIAMETER	1 Very thin (< 4 mm) 3 Thin (4 - 6 mm) 5 Intermediate (7 - 9 mm) 7 Thick (10 - 12 mm) 9 Very thick (> 12 mm)	M A T U R E TYPE OF LEAF LOBES	0 No lateral lobes (entire) 1 Very slight (teeth) 3 Slight 5 Moderate 7 Deep 9 Very deep	FOLIAGE COLOR OR INMATURE I	1 Yellow-green 2 Green 3 Green with purple edge 4 Greyish (heavy pubescence) 5 Green with purple veins on upper surface 6 Slightly purple 7 Moderately purple 8 Mostly purple 9 Totally purple
PLANT TYPE	3 Erect (less than 75 cm) 5 Semi-compact (75 - 150 cm) 7 Spreading (151 - 250 cm) 9 Extremely spreading (more than 250 cm)	GENERAL OUTLINE OF THE LEAF	1 Rounded 2 Reniform 1 3 Cordate 3 4 Triangular 5 Hastate 6 Lobed 7 Almost divided	FOLI MATURE LEAF COLOR	1 Yellow-green 2 Green 3 Green with purple edge 4 Greyish (heavy pubescence) 5 Green with purple veins on upper surface 6 Slightly purple 7 Moderately purple 8 Mostly purple 9 Totally purple
TWINING	0 Non-twining 3 Slighty twining 5 Moderately twining 7 Twining 9 Very twining	VINE TIP PUBESCENCE	0 None 3 Sparse 5 Moderate 7 Heavy 9 Very heavy	MATURE LEAF SIZE	3 Small (< 8 cm) 5 Medium (8 - 15 cm) 7 Large (16 - 25 cm) 9 Very large (> 25 cm)

STORAGE	STORAGE ROOT SHAPE	STORAC	STORAGE ROOT DEFECTS	STORA	STORAGE ROOT CORTEX THICKNESS
1 Round 2 Round elliptic 3 Elliptic 4 Ovate 5 Obovate 6 Oblong 7 Long oblong 8 Long elliptic 9 Long irregular or curved	Q	0 None 1 Alligator-like skin 2 Veins 3 Shallow horizontal constrictions 4 Deep horizontal constrictions 5 Shallow longitudinal grooves 6 Deep longitudinal grooves 7 Deep constrictions and deep grooves	constrictions nstrictions Il grooves rooves and deep grooves	1 Very thin (1 mm or less) 3 Thin (1 - 2 mm) 5 Intermediate (2 - 3 mm) 7 Thick (3 - 4 mm) 9 Very thick (more than 4 mm)	less) mm) ıan 4 mm)
PREDOMINANT COLOR	STORAGE ROOT SKIN COLOR INTENSITY	SECONDARY COLOR	PREDOMINANT COLOR	STORAGE ROOT FLESH COLOR SECONDARY COLOR	OLOR DISTRIBUTION OF COLOR
1 White 2 Cream 3 Yellow 4 Orange 5 Brownish orange 6 Pink 7 Red 8 Purple-red 9 Dark purple	1 Pale 2 Intermediate 3 Dark	0 Absent 1 White 2 Cream 3 Yellow 4 Orange 5 Brownish orange 6 Pink 7 Red 8 Purple-red 9 Dark purple	1 White 2 Cream 3 Dark cream 4 Pale yellow 5 Dark yellow 6 Pale orange 7 Intermediate orange 8 Dark orange 9 Strongly pigmented with anthocyanins	0 Absent 1 White 2 Cream 3 Yellow 4 Orange 5 Pink 6 Red 7 Purple-red 8 Purple	0 Absent 1 Narrow ring in cortex 2 Broad ring in cortex 3 Scattered spots 4 Narrow ring in flesh 5 Broad ring in flesh 6 Ring and other areas 7 In longitudinal sections 8 Covering most flesh 9 Covering all flesh

STORAGE ROOT ARRANGEMENT

1 Closed cluster 3 Open cluster 5 Disperse 7 Very disperse



The International Potato Center (CIP) is a scientific, autonomous, and non-profit institution dedicated to develop and disseminate knowledge for greater use of the potato and other tuber and root crops as basic foods in the developing world. CIP was established by agreement with the Government of Peru and is supported by the Consultative Group on International Agricultural Research (CGIAR) whose members provide funding for international agricultural development.

El Centro Internacional de la Papa (CIP) es una institución científica, autónoma y sin fines de lucro, dedicada a desarrollar y diseminar conocimientos sobre la papa y otros cultivos de tubérculos y raíces, para lograr su mayor utilización como alimentos básicos en los países en desarrollo. El CIP fue establecido mediante convenio con el Gobierno del Perú y es apoyado por el Grupo Consultivo sobre Investigaciones Agronómicas Internacionales (GCIAI) cuyos miembros proveen fondos para el desarrollo agrícola internacional.

Le Centre International de la Pomme de Terre (CIP) est une institution scientifique, autonome et sans but lucratif, dont l'objectif est de développer et diffuser des connaissances pour mieux tirer profit de la pomme de terre et autres tubercules et racines comme aliments de base dans les pays en voie de développement. Le CIP a été établi avec l'accord du Gouvernement du Pérou et a l'appui du Groupe Consultatif pour la Recherche Agricole Internationale (GCRAI) dont les membres fournissent des fonds pour le développement agricole international.