Sweetpotato Germplasm Management (*Ipomoea batatas*)

Training manual

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Section 1.1

Systematic botany and morphology of the sweetpotato plant

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The sweetpotato is a plant that was probably originated in or near northwestern South America. The most common names for this plant in Latin America are **batata, camote**, **boniato, batata doce, apichu,** and **kumara**.

The systematic classification of the sweetpotato is as follows:

Family: **Convolvulaceae**
Tribe: **Ipomoeae**
Genus: **Ipomoea**
Sub-genus: **Eriospermum**
Section: **Eriospermum**
Series: **Batatas**
Species: ***Ipomoea batatas*** **(L.) Lam.**

This species was first described in 1753 by Linnaeus as *Convolvulus batatas*. However, in 1791 Lamarck classified this species within the genus *Ipomoea* on the basis of the stigma shape and the surface of the pollen grains. Therefore, the name was changed to *Ipomoea batatas* (L.) Lam.

Within the Series Batatas there are 13 wild species that are considered to be related to the sweetpotato (Austin, 1978; 1979; Austin and Huaman, 1996). These are:

I. cordatotriloba (= *I. trichocarpa)*
I. cynanchifolia
I. grandifolia
I. lacunosa
I. x leucantha
I littoralis
I. ramosissima
I. tabascana
I. tenuissima
I. tiliacea
I. trifida
I. triloba
I. umbraticola

Two species that used to be considered within this group were *I. peruviana* from Peru and Ecuador, now classified within section *Eriospermum,* series *Setosae*, and *I. gracilis* from Australia, now in section *Erpipomoea* (McDonald and Austin, 1990).

According to Austin (1978), two of these species are considered to be of hybrid origin. *I. x leucantha* has been determined to be intermediate *I. cordatotriloba* x *I. lacunosa* hybrids, and *I. x grandifolia* has been hypothesized to include derivatives of *I. cordatotriloba* x *I. batatas* hybrids.

The number of chromosomes in the sweetpotato plant is 2n = 6x = 90. This indicates that it is a hexaploid plant with a basic chromosome number x = 15. Among the wild species, *I. tabascana* and *I. tiliacea* are tetraploids with 2n = 4x = 60. The other species are diploids with 2n = 2x = 30. Polyploid species are *I. cordatotriloba* with 2x and 4x and *I. trifida* with 2x, 3x, 4x, and 6x (Huamán and Zhang, 1997).

The geographic distribution of the wild species of Series Batatas is within the Americas. This is with exception of *I. littoralis* that is found in Australia and Asia.

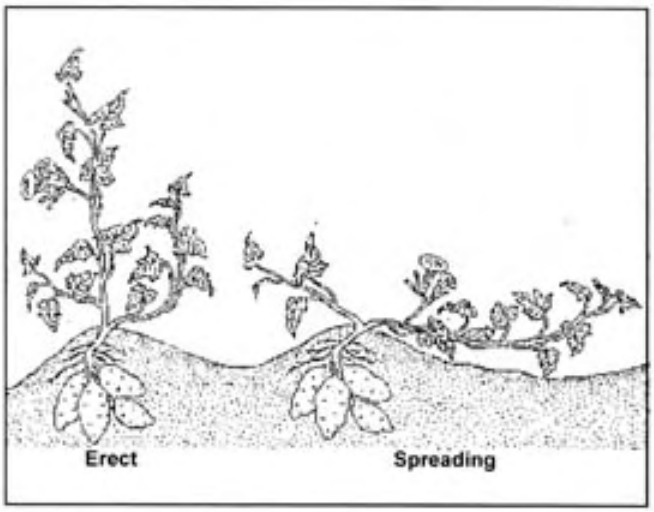
The cultivated species *I. batatas* includes plants that are very variable in their morphology. Thousands of cultivars have been selected and cultivated in Latin America since ancient times. At the present time, it

is cultivated throughout the tropics. However, the largest plantings of sweetpotatoes are found in China and other countries of Asia.

Additional information to the following description of the sweetpotato morphology can be found in Bartolini, 1985; Hayward, 1967; Kays, 1985.

Growth habit

The sweetpotato is a herbaceous and perennial plant. However, it is grown as an annual plant by vegetative propagation using either storage roots or stem cuttings. Its growth habit is predominantly prostrate with a vine system that expands rapidly horizontally on the ground. The types of growth habit of sweetpotatoes are erect, semi-erect, spreading, and very spreading.



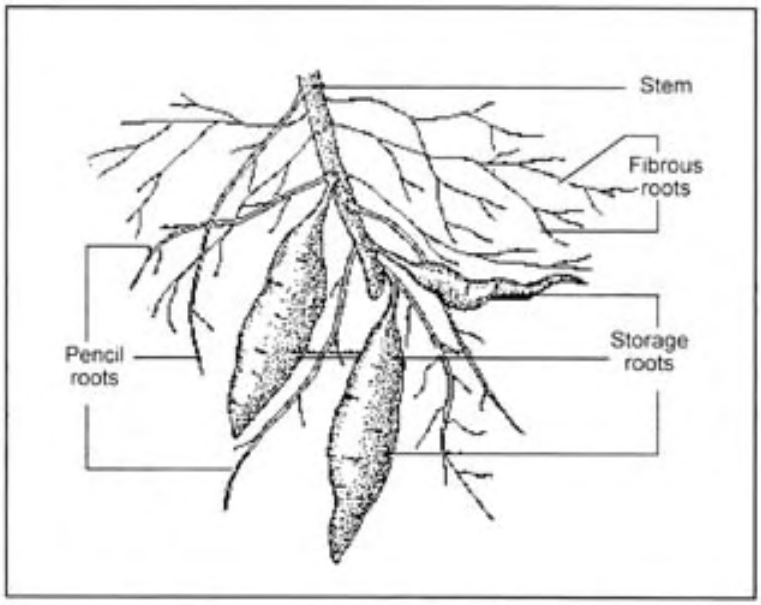
Types of growth habits in sweetpotatoes.

Root system

The sweetpotato root system consists of **fibrous roots** that absorb nutrients and water, and anchor the plant, and **storage roots** that are lateral roots, which store photosynthetic products.

The root system in plants obtained by vegetative propagation starts with adventitious roots that develop into primary **fibrous roots**, which are branched into lateral roots. As the plant matures, thick **pencil roots** that have some lignification are produced. Other roots that have no lignification, are fleshy and thicken a lot, are called **storage roots**.

Plants grown from true seed form a typical root with a central axle with lateral branches. Later on, the central axle functions as a storage root.

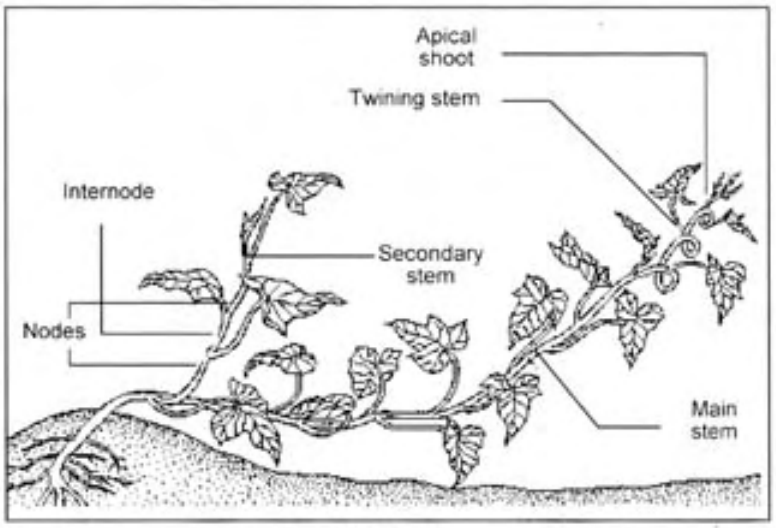


Types of roots in the sweetpotato plant .

Stem

A sweetpotato stem is cylindrical and its length, like that of the internodes, depends on the growth habit of the cultivar and of the availability of water in the soil. The erect cultivars are approximately 1 m long, while the very spreading ones can reach more than 5 m long. Some cultivars have stems with twining characteristics. The internode length can vary from short to very long, and, according to stem diameter, can be thin or very thick.

Depending on the sweetpotato cultivar, the stem color varies from green to totally pigmented with anthocyanins (red-purple color). The hairiness in the apical shoots, and in some cultivars also in the stems, varies from glabrous (without hairs) to very pubescent.

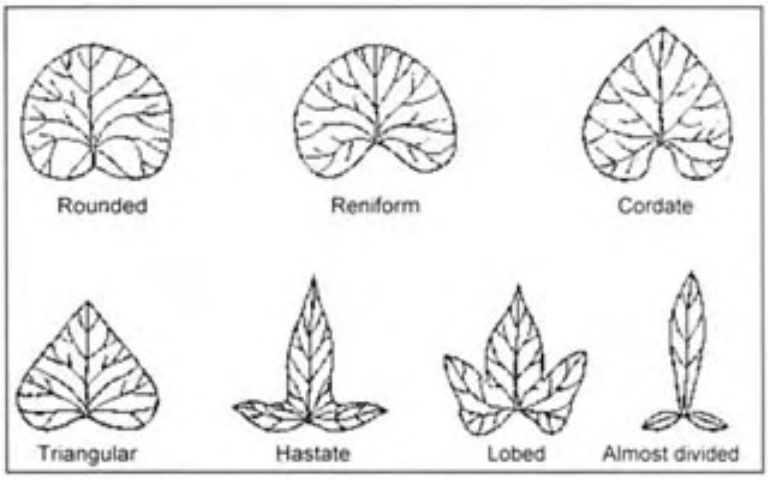


Parts of the stem.

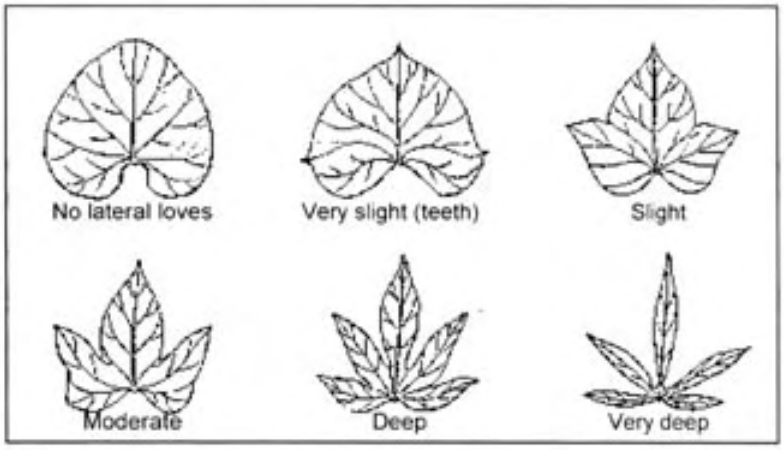
Leaves

The leaves are simple and spirally arranged alternately on the stem in a pattern known as 2/5 phyllotaxis (there are 5 leaves spirally arranged in 2 circles around the stem for any two leaves be located in the same vertical plane on the stem).

Depending on the cultivar, the edge of the leave lamina can be entire, toothed or lobed. The base of the leaf lamina generally has two lobes that can be almost straight or rounded. The shape of the general outline of sweetpotato leaves can be rounded, reniform (kidney-shaped), cordate (heart-shaped), triangular, hastate (trilobular and spear-shaped with the two basal loves divergent), lobed and almost divided. Lobed leaves differ in the degree of the cut, ranging from superficial to deeply lobed. The number of lobes generally range from 3 to 7 and can be easily determined by counting the veins that go from the junction of the petiole up to the edge of the leaf lamina. However, toothed leaves have minute lobes called teeth which could number from 1 to more than 9. Some cultivars show some variation in leaf shape on the same plant.



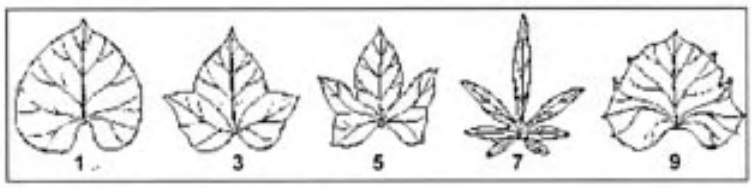
General outline of the leaf.



Types of leaf lobes.

The leaf color can be green-yellowish, green or can have purple pigmentation in part or all the leaf blade. Some cultivars show purple young leaves and green mature leaves. The leaf size and the degree of hairiness vary according to the cultivar and environmental conditions. The hairs are glandular and generally are more numerous in the lower surface of the leaf. The leaf veins are palmated and their color, which is very useful to differentiate cultivars, can be green to particularly or totally pigmented with anthocyanins.

The length of the petiole ranges from very short to very long. Petioles can be green or with purple pigmentation at the junction with the lamina and/or with the stem or throughout the petiole. On both sides of the insertion with the lamina there are two small nectaries.



Number of leaf lobes.

Flowers

Sweetpotato cultivars differ in their ability of flower. Under normal conditions in the field, some cultivars do not flower, others produce very few flowers, and others flower profusely.

The inflorescence is generally a cyme in which the peduncle is divided in two axillary peduncles; each one is further divided in two after the flower is produced (biparous cyme). In general, buds of first, second, and third order are developed. However, single flowers are also formed. The flower buds are joined to the pedundle through a very short stalk called pedicel. The color of the flower bud pedicel, and peduncle varies from green to completely purple pigmented.

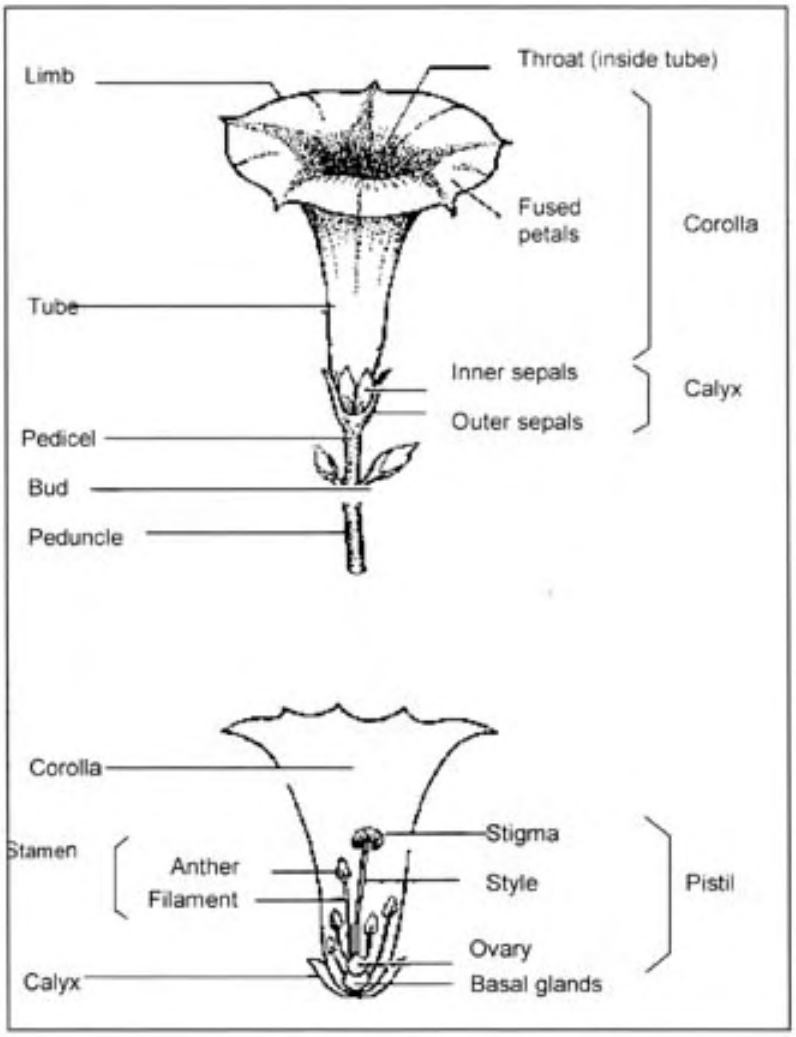
The flower is bisexual. Besides the calyx and corolla, they contain the stamens that are the male organs or androecium and the pistil that is the female organ or gynoecium.

The calyx consists of 5 sepals, 2 outer and 3 inner, that stay attached to the floral axle after the petals dry up and fall.

The corolla consists of 5 petals that are fused forming a funnel, generally with lilac or pale purple limb and with reddish to purple throat (the inside of the tube). Some cultivars produce white flowers.

The androecium consists of five stamens with filaments that are covered with glandular hairs and that are partly fused to the corolla. The length of the filaments is variable in relation to the position of the stigma. The anthers are whitish, yellow or pink, with a longitudinal dehiscence. The pollen grains are spherical with the surface covered with very small glandular hairs.

The gynoecium consists of a pistil with a superior ovary, two carpels, and two locules that contain one or two ovules. The style is relatively short and ends in a broad stigma that is divided into two lobes that are covered with glandular hairs. At the base of the ovary there are basal yellow glands that contain insect-attracting nectar. The stigma is receptive early in the morning and the pollination is mainly by bees.



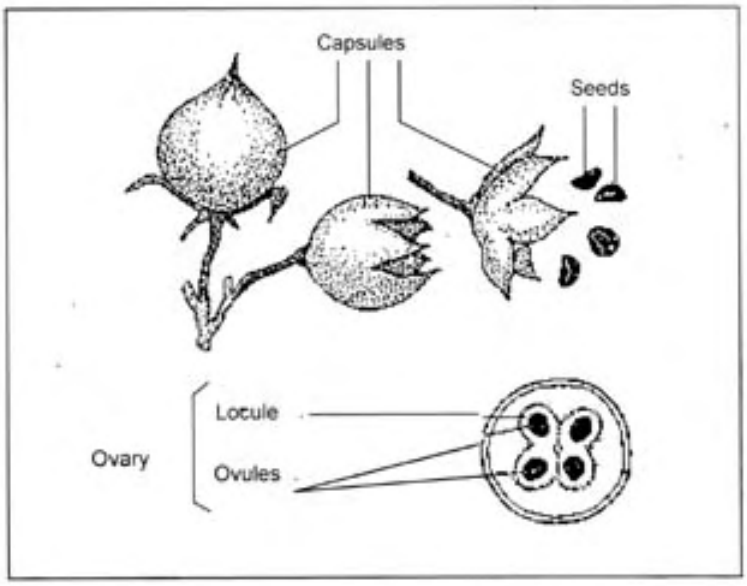
Parts of the flower.

Fruit and seeds

The fruit is a capsule, more or less spherical with a terminal tip, and can be pubescent or glabrous. The capsule turns brown when mature.

Each capsule contains from one to four seeds that are slightly flattened on one side and convex on the other. Seed shape can be irregular, slightly angular or rounded; the color ranges from brown to black; and the size is approximately 3mm. The embryo and endosperm are protected by a thick, very hard and impermeable testa. Seed germination is difficult and requires scarification by mechanical abrasion or chemical treatment. Sweetpotato seeds do

not have a dormancy period but maintain their viability for many years.

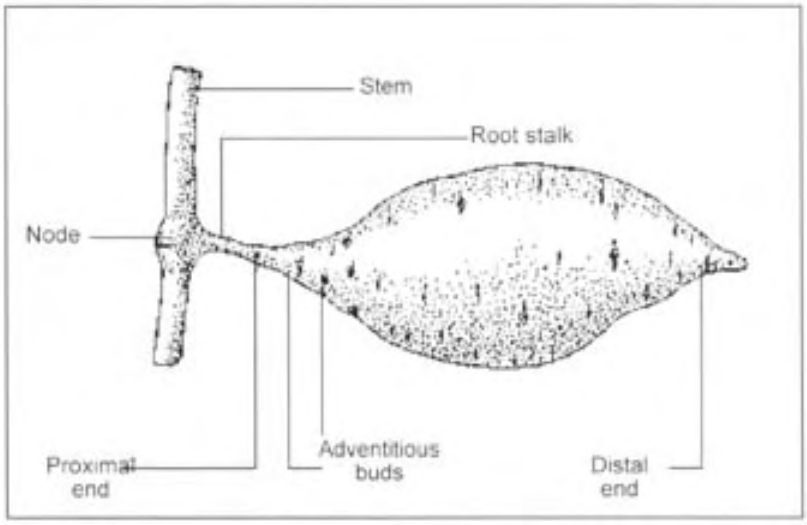


The fruit is a capsule with one to four seeds.

Storage root

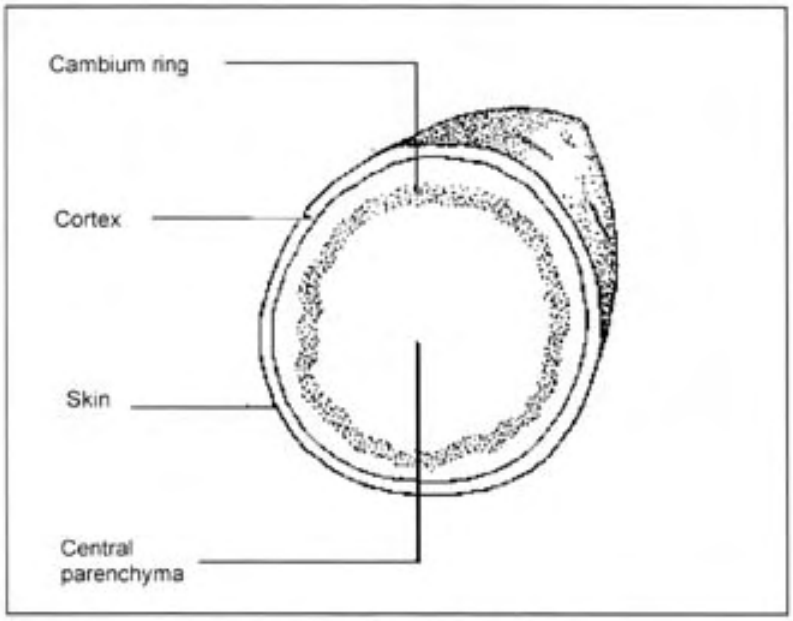
The storage roots are the commercial part of the sweetpotato plant, and sometimes are mistakenly named "tubers". Most cultivars develop storage roots at the nodes of the mother stem cuttings that are underground. However, the very spreading cultivars produce storage roots at some of the nodes that come into contact with the soil.

The parts of the storage roots are the proximal end that joins to the stem, through a root stalk, and where many adventitious buds are found from which the sprouts are originated; a central part which is more expanded; and the distal end that is opposite to the root stalk. The adventitious buds that are located in the central and distal part usually sprout later than those located in the proximal end.



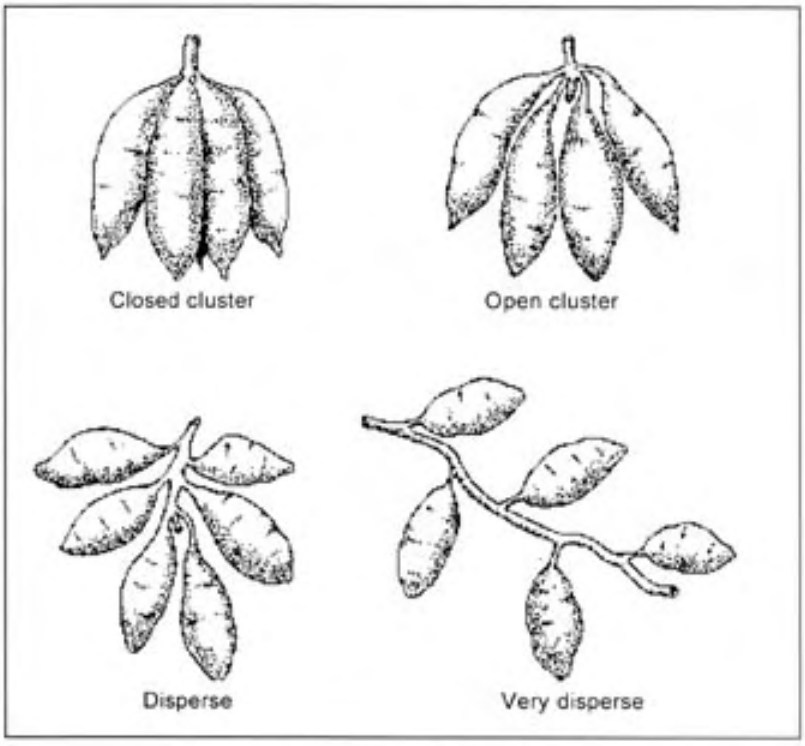
Parts of the storage roots

A transverse section of the storage roots shows the protective periderm or skin, the cortex or cortical parenchyma that, depending on the cultivar, varies from very thin to very thick, the cambium ring where the latex vessels are found, and the medulla or central parenchyma. The amount of the latex formed depends on the maturity of the storage root, the cultivar, and the soil moisture during the growing period. The latex drops are produced when the storage roots are cut and they darken very quickly due to the oxidation.

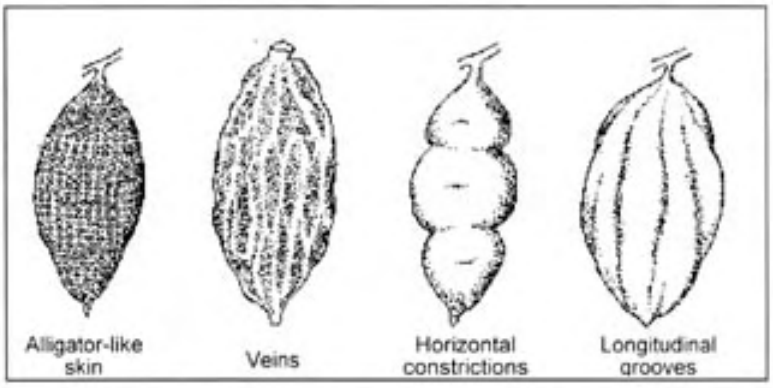


Parts inside the storage roots.

The formation of the storage roots can be in clusters around the stem. If the rootstalk that joins the root to the stem is absent or is very short, it forms a closed cluster. If the stalk is long, it forms an open cluster. In some other cultivars, the storage roots are formed at a considerable distance from the stem and therefore, the storage root formation is disperse or very disperse.



Types of storage root formation.

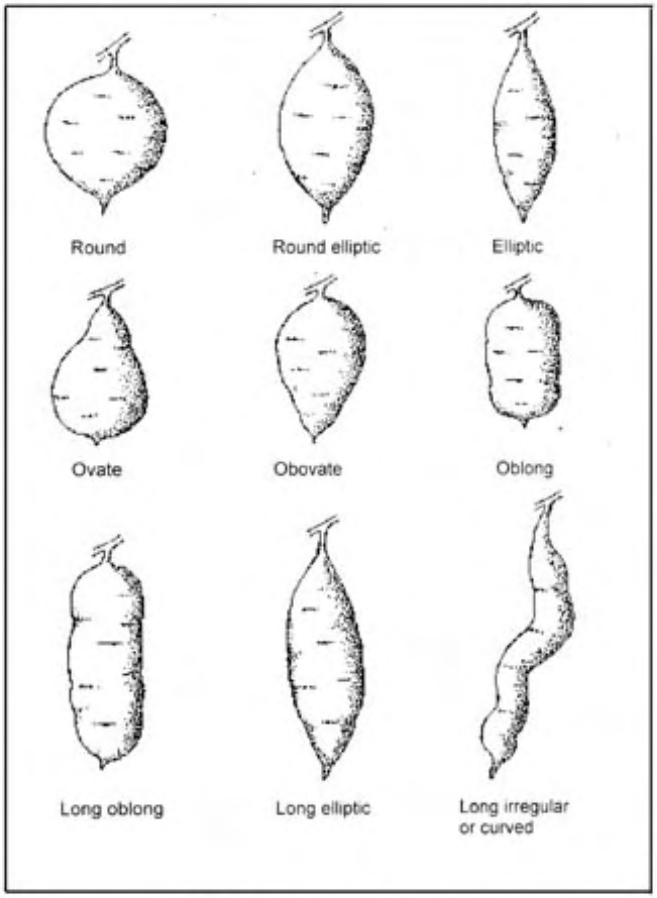


Types of defects on the storage root surface.

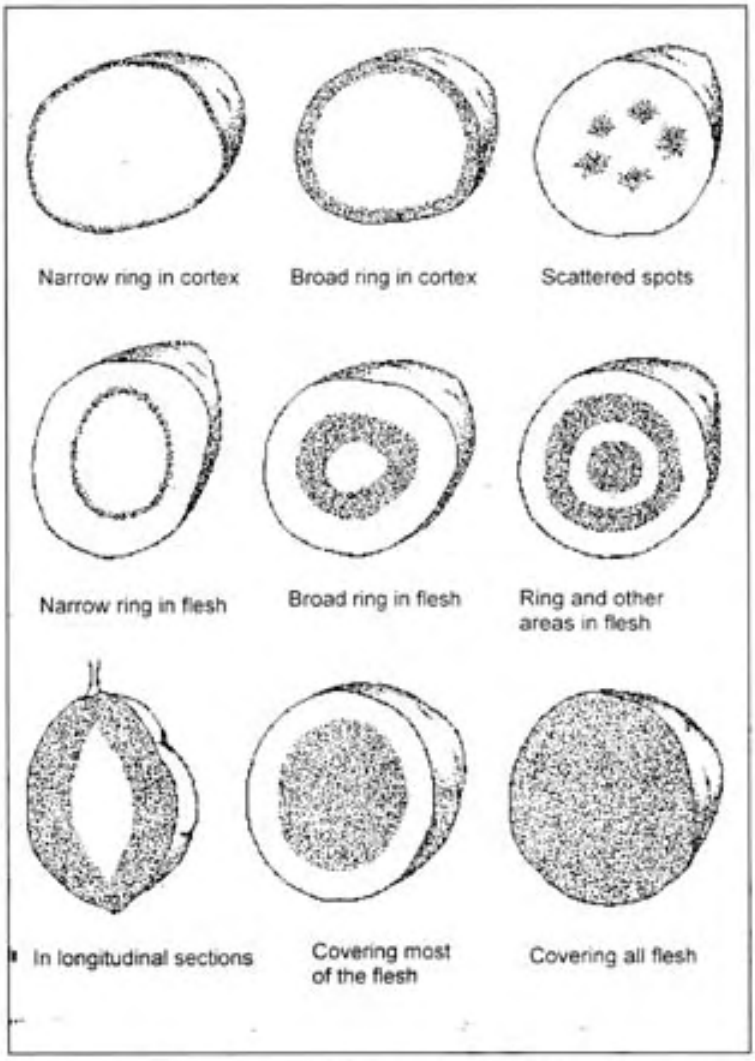
The storage root surface is usually smooth but some cultivars show some deffects such as alligator-like skin, prominent veins, horizontal constrictions or longitudinal grooves. Lenticels are also located on the surface and in some cultivars they can be protuberant due to excess water in the soil.

Storage roots vary in shape and size according to the cultivar, type of soil where the plant is grown and other factors. The outline of their shape can be round, round-elliptic, elliptic, ovate, obovate, oblong, long oblong, long elliptic, and long irregular or curved.

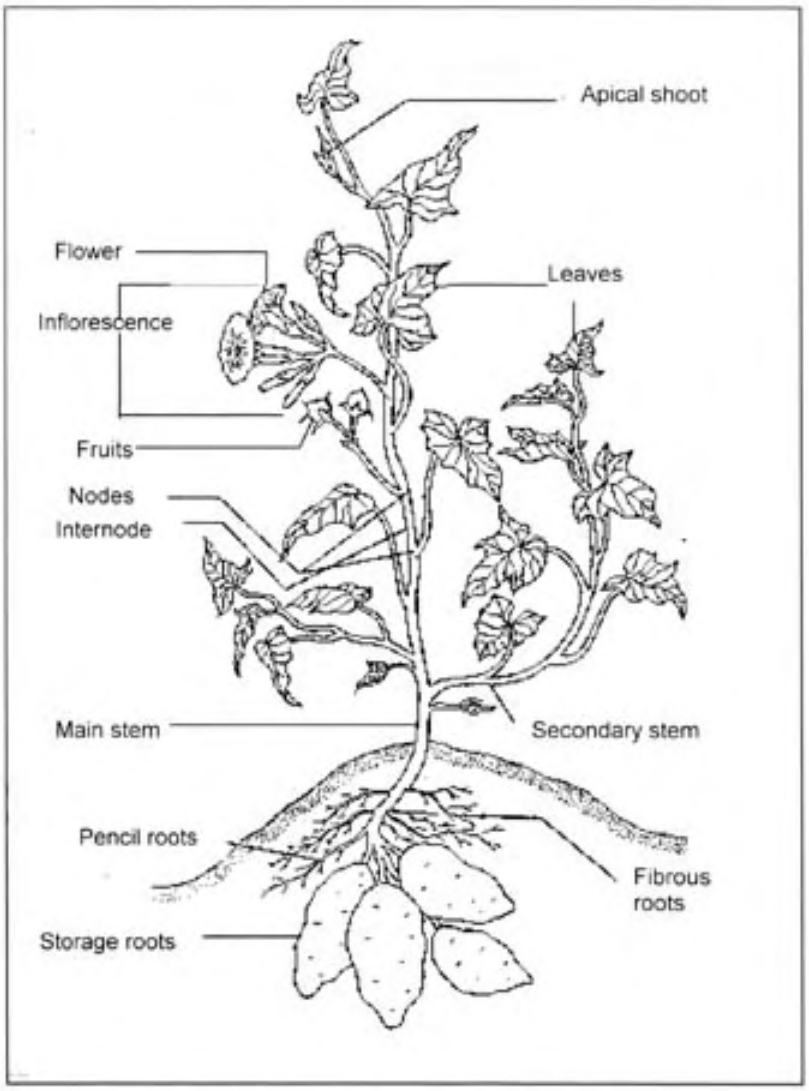
The storage root skin color can be whitish, cream, yellow, orange, brown-orange, pink, red-purple, and very dark purple. The intensity of the color depends on the environmental conditions where the plant is grown. The flesh color can be white, cream, yellow, or orange. However, some cultivars show red-purple pigmentation in the flesh in very few scattered spots, pigmented rings or, in some cases, throughout the entire flesh of the root.



Types of storage root shapes.



Distribution of anthocyanin pigmentation in the storage root flesh .



Morphology of the sweetpotato plant.

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Section 1.2

Botany, origin, evolution and biodiversity of the sweetpotato

Z. Huamán

Introduction

Sweetpotato is the world's seventh most important food crop after wheat, rice, maize, potato, barley, and cassava. It is grown in more developing countries than any other root crop. World sweetpotato production is around 124 million tons in an area of about 9.2 million hectares. China is the world's leading sweetpotato-producing country with about 90% of production. Of the 82 developing countries where sweetpotatoes are grown in Africa (36), Asia (22), and Latin America (24), 40 countries count sweetpotato among the five most important food crops produced on an annual basis. Japan and the United States are among the few industrialized countries that grow sweetpotatoes

today (CIP, 1996). The crop is cultivated in the tropics, subtropics, and warmer temperate zones in latitudes between about 40° N and 40° S and from sea level to about 2000 m elevation in tropical highland zones.

Botany

The sweetpotato belongs to a single species, *Ipomoea batatas* (L.) Lam. In Spanish, the most common names are batata, camote, and boniato; in French, patate douce; in Portuguese, batata doce; in Italian, batata dolce; in Chinese, gan shu; and in Quechua, the Inca language, kumara or apichu.

Linnaeus described the cultivated sweetpotato in 1753 as *Convolvulus batatas*. In 1791, the botanist Lamarck described it as *Ipomoea batatas*. It is a hexaploid plant with 2n=6x=90 chromosomes, although some plants morphologically quite similar to *I. batatas* with 2n=4x=60 have been described and named, but they are considered synonyms of this species (Austin, 1977). *I. batatas* is a self-incompatible species.

The sweetpotato and the wild species closely related to it are classified in the family Convolvulaceae, genus Ipomoea, subgenus Eriospermum, section Eriospermum (formerly Batatas), and series Batatas (Austin and Huamán, 1996).

The genus Ipomoea comprises 600 to 700 species. Over half of the species are concentrated in the Americas, where there may be 400 taxa, classified within the subgenera Eriospermum, Quamoclit, and Ipomoea. These three subgenera contain 10 sections, seven of which were originally confined to the Americas before the species were dispersed as cultigens, medicinal plants, and weeds (Austin and Huamán, 1996).

Subgenus Eriospermum, section Eriospermum, series Batatas contains, in addition to *I. batatas*, 13 wild species closely related to the sweetpotato (Table 1). All of these species except *I. littoralis* are endemic to the Americas. Two are considered to be of hybrid origin. I. x leucantha has been determined to be intermediate *I. cordatotriloba* x *I. lacunosa* hybrids, and I. x grandifolia has been hypothesized to include derivatives of *I. cordatotriloba* x *I. batatas* hybrids (Austin, 1978). Two species that used to be considered within this group were *I. peruviana* from Peru and Ecuador, now classified within section Eriospermum, series Setosae, and *I. gracilis* from Australia, now in section Erpipomoea.

Table 1. Ploidy 1 and compatibility of Ipomoea species, section Eriospermum, series Batatas.

Ipomoea species	Ploidy 2n	Compatibility
	x=15	
I. cynanchifolia	2x=30	C
I. lacunosa	2x=30	C
I. x leucantha	2x=30	C3
I. littoralis 2	2x=30	I
I. ramosissima	2x=30	C
I. tenuissima	2x=30	C
I. triloba	2x=30	C
I. umbraticola 2	2x=30	C
I. tiliacea	4x=60	I
I. tabascana 2	4x=60	C
I. cordatotriloba 2 (before *I. trichocarpa)*	2x, 4x	C
I. trifida	2x, 3x,4x,6x	I
I. grandifolia	2x ?	C3
I. batatas	6x=90	I

1. According to Jones, 1974; Nishiyama et al., 1975; Austin, 1988
2. According to Jarret et al., 1992, Ozias-Akins y Jarret, 1994.
3. CIP's data

C = self-compatible, I = self-incompatible, (Nishiyama et al.,1975).

Origin

It is generally accepted that the sweetpotato is of American origin. Abundant evidence shows that sweetpotato was spread widely through the migration routes of people in the New World tropics before the discovery of America. Two main groups of sweetpotato were known during that period. The **aje** (an Arawakan word) group, which was starchy and had a slightly sweet taste, and the batata group, which was also starchy but markedly sweet in taste (Austin, 1988). O'Brien (1972) showed linguistic and historical evidence indicating that this crop had reached southern Peru and southern Mexico by about 2000 to 2500 BC.

Linguistic evidence has also shown three lines of dispersal of the sweetpotato from America. The **kumara** line is prehistoric and based on lexical parallels between the Quechua name and Polynesian word kumara. This could explain the transfer of sweetpotato by Peruvian or Polynesian voyagers from northern South America to eastern

Polynesia around AD 400. The **batata** line dates from the first voyage of Columbus in 1492, which resulted in the introduction of West Indian sweetpotatoes to Western Europe. Portuguese explorers transferred sweetpotatoes grown in western Mediterranean Europe to Africa, India and the East Indies in the 16th century. The sweetpotato was recorded in South China by 1594 and in southern Japan by 1698. The **camote** (name derived from the word **camotli** in the Mayan language Nahuatl) line represents the direct transfer of Mexican sweetpotatoes by Spanish trading galleons between Acapulco and Manila, Philippines, in the 16th century (Yen, 1982).

Austin (1988) postulated that the center of origin of *I. batatas* was somewhere between the Yucatan peninsula of Mexico and the mouth of the Orinoco River in Venezuela, where *I. trifida* and *I. triloba* might have been crossed and might have produced the wild ancestor of *I. batatas*. Native people in the area may have discovered the sweetpotato and brought it into cultivation. By at least 2500 BC, the cultigen had most likely been spread by the Mayas and Incas to almost the limits for cultivation in Central and South America that existed at the time when the Europeans arrived. Carbon-dated sweetpotatoes discovered in the Chilca canyon on the coast of Peru were estimated to be from 8000 to 10000 years before present, which indicates that sweetpotato may be among the world's earliest domesticates (Engel, 1970; Yen, 1974).

The primary center of diversity of sweetpotatoes is located in northwestern South America (Colombia, Ecuador and Peru) and parts of Central America (such as Guatemala) where a great diversity of native sweetpotatoes, weeds, and wild Ipomoea exists. Secondary centers of sweetpotato diversity outside of the Americas are in China, Southeast Asia, New Guinea and East Africa (Austin 1983, 1988; Yen 1982). Sweetpotato germplasm found outside the Americas, however, has been reported to contain only a small sample of the Latin American variability (Yen, 1974).

Evolution

I. batatas is not known in the wild state and plants found growing as wild plants are remnants from abandoned cultivated fields or plants coming from sweetpotato seeds, which continue growing by vegetative propagation. Several wild Ipomoea species having some morphological resemblance to *I. batatas* have been considered as potential wild ancestors of the sweetpotato. One of these species is *I. trifida*, which Nishiyama (1963) collected in Mexico and reported as a 6x *I. trifida* (accession K123) and claimed to be morphologically similar to *I. batatas*, except that it produced only slightly swollen storage roots. A. Jones (1967) demonstrated that K123 could be an *I. batatas* derivative found growing in the wild. He pointed out that other characteristics considered by Nishiyama as typical of wild plants, such

as the twining habit, are also observed in sweetpotatoes and that many genotypes derived from sweetpotato seeds do not produce storage roots. Furthermore, he reported that F1 (K123 x *I. batatas*) hybrids produce abundant seeds and the chromosome pairing in metaphase I of the hybrids is similar to that of crosses between sweetpotatoes.

It is not yet defined whether *I. batatas* is an allopolyploid or an autopolyploid, but sexual polyploidization through the production of unreduced gametes might have facilitated the evolution of *I. batatas* to the hexaploid level. The formation of unreduced pollen has been reported in diploid *I. trifida* (Orjeda et al., 1990) and in some tetraploid and hexaploid *I. batatas* (Bohac et al., 1992). Freyre et al. (1991) also reported 2n-egg production in 3x *I. trifida* that generated 6x genotypes in their progenies. Nishiyama (1971) and Austin (1988) suggested an allopolyploid origin and that *I. trifida* is one of the species most closely related to the sweetpotato. Nishiyama (1971) proposed that sweetpotato might have originated from 2x *I. leucantha*, which produced 4x *I. littoralis*; 2x x 4x crosses between these two species might have originated 3x *I. trifida*, from which 6x *I. trifida* were derived. Further selection and domestication of these wild plants might have originated 6x *I. batatas*. Nishiyama et al. (1975) reported similarities in some plant characters, sexual compatibility, and behavior between sweetpotato and artificial 6x hybrids produced from *I. leucantha* and *I. littoralis*. Based on numerical analysis of key morphological characters, Austin (1988) hypothesized that *I. triloba* and *I. trifida* are the species that contributed the sweetpotato genome. He also considered that *I. tiliacea* might have been involved in the origin of sweetpotato. With cytogenetical evidence, Shiotani (1988) proposed that sweetpotato has the genomic structure of an autohexaploid with the B genome that also exists in autotetraploids and diploids of the *I. trifida* complex.

Biodiversity

Most countries in Latin America now consider sweetpotato of minor importance mainly because its use as a food has decreased considerably. Farmers in most Latin American and Caribbean countries also indicate that many sweetpotato cultivars have disappeared because the crop's use as a food has declined. The emphasis placed on the use of early maturing cultivars to fit in crop rotation systems of modern agriculture has also certainly caused genetic erosion of very late maturing sweetpotatoes. In countries where sweetpotato breeding programs existed, new bred varieties have replaced many native cultivars. In Argentina, for example, cultivar Morada INTA is the most widely grown sweetpotato in most of the country. In Peru, cultivars Nemañete, María Angola, and Japones Tresmesino have replaced native cultivars in the valleys of the central coast.

Since 1985, CIP has carried out 90 collecting expeditions with active participation of NARS of 16 countries in Latin America and the Caribbean. Sweetpotato genetic resources collected included a total of 1,157 wild accessions: 532 accessions of 11 wild species in series Batatas (Table 2); 419 accessions of 52 other wild species, and 206 accessions of other wild or weedy materials.

Table 2. Geographic distribution of wild Ipomoea species, series Batatas, conserved in the genebank maintained at CIP

Ipomoea species	Number of samples	Geographical distribution
I. cordatotriloba (before *I. trichocarpa*	90	ARG (18); BOL (3);COL (1); MEX (2); PRY (66)
I. cynanchifolia	3	BRA(3)
I. grandifolia	126	ARG(79); BRA(2); PRY(39);URY(6)
I. x leucantha	14	ARG(4); COL(5); ECU(2); MEX(1); PER(1); VEN(1)
I. tabascana	1	MEX(1)
I. tiliacea	41	CUB(20); DOM(5); GTM(1); JAM(6); MEX(2); NIC(7)
I. trifida	170	COL(24); CUB(8); ECU(3); GTM(53); MEX(2); NIC(56); VEN(24)
I. triloba	51	COL(13); CUB(7); DOM(4); ECU(4); MEX(6); PER(10); PRY(1); VEN(6)
I. ramosissima	31	ARG(3); BOL(6); COL(2); ECU(1); NIC(2); PER(17)
I. umbraticola	5	MEX(2); NIC(3)
I. lacunosa	0	North America
I. littoralis	0	Asia
I. tenuissima	0	The Caribbean
Total	532	

During those collecting expeditions, abundant samples of sweetpotato cultivars native to Latin America were also obtained (Huamán and De la Puente, 1988). The cultivated collection at CIP was further expanded by donations of smaller collections from other countries, the transfer of sweetpotato collections maintained in other international centers such as AVRDC in Taiwan and IITA in Nigeria, and donations of breeding lines or advanced cultivars from several countries. The genebank at CIP now maintains a total of 5,526

cultivated accessions (Table 3), comprising 4,168 accessions of native and advanced cultivars from 57 countries (22 in the Americas, 26 in Asia, and 9 in Africa), and 1,358 breeding lines. A number of institutions in Latin America, Asia, and Africa also maintain national sweetpotato collections. Those in Latin America have been organized with duplicate samples of sweetpotato collected jointly with CIP and left with the national institution designated as the country's germplasm repository.

Altitude of the sites where sweetpotatoes have been collected ranges from 0 to 3,000 m. In Latin America, sweetpotatoes have been found at 1,900-2,500 m in Bolivia, Colombia, and Venezuela, and at up to 3,00 m in Ecuador and Peru. In Asia, sweetpotatoes have been found growing from 1,900 to 2,700 m only in New Guinea.

Because of the asexual propagation of sweetpotato cultivars, numerous duplicate accessions of the same cultivar have been found in the cultivated collection maintained at CIP. There is an ongoing effort to identify these duplicate accessions. A revised version of the list of sweetpotato descriptors has been used (CIP/ AVRDC/ IBPGR, 1991) to select key morphological descriptors that adequately describe each accession. A color chart for the characterization of storage-root skin and flesh color has also been produced to obtain more consistent data. Computerized systems for multivariate analyses are used to group morphologically similar accessions that are grown side by side in the field. Accessions that turn out to be morphologically identical are also compared by molecular markers like random amplified polymorphic DNA (RAPD) fingerprints. Those that are morphologically identical and produce the same DNA fingerprints are considered duplicates. One accession from each duplicate group is selected to represent the group on the basis of its data on reaction to pests and diseases. All duplicate samples are then converted into true seed and their clonal forms discarded. By using this methodology, the number of Peruvian accessions in the collection was reduced from 1,939 to 673. The number of duplicates of the same cultivar ranged from 1 to 99 accessions. A Peruvian sweetpotato core collection comprising 85 accessions (12% of 673) was selected to enhance the utilization of this germplasm. (Huamán et al.,1999).

Table 3. Geographic coverage of *Ipomoea batatas* conserved in the genebank maintained at CIP

Area and country	Number of accessions	Country	Number of accessions
America			
Argentina (ARG)	106	Bolivia (BOL)	78
Brazil (BRA)	149	Chile (CHL)	1
Colombia (COL)	174	Ecuador (ECU)	172
Peru (PER)	1,099	Paraguay (PRY)	73
Uruguay (URY)	2	Venezuela (VEN)	86
Panama (PAN)	47	Costa Rica (CRI)	40
Nicaragua (NIC)	11	Honduras (HND)	8
Cuba (CUB)	207	Dominican Republic (DOM)	114
Jamaica (JAM)	52	Puerto Rico (PRI)	38
Saint Vincent (VCT)	10	Guatemala (GTM)	100
Mexico (MEX)	22	United States (USA)	212
Asia			
Bangladesh (BGD)	4	Burma (BUR)	3
China (CHN)	38	Morocco (MAR)	1
Hong Kong (HKG)	1	Korea (KOR)	10
Indonesia (IDN)	31	Japan (JPN)	142
Lao Peoples Republic (LAO)	8	Malaysia (MYS)	12
Philippines (PHL)	51	Papua New Guinea (PNG)	474
Singapore (SGP)	3	Sri Lanka (LKA)	5
Taiwan (TWN)	324	Thailand (THA)	94
Vietnam (VNM)	2	Australia (AUS)	3
Cook Islands (COK)	6	Fiji (FJI)	4
New Caledonia (NCL)	2	New Hebrides (NHB)	2
New Zealand (NZL)	7	Solomon Islands (SLB)	63
Tonga (TON)	18	Niue (NIU)	5
Africa			
Burundi (BDI)	5	Cameroon (CMR)	4
Egypt (EGY)	2	Madagascar (MDG)	2
Kenya (KEN)	2	Nigeria (NGA)	18
Rwanda (RWA)	4	South Africa (ZAF)	2
Uganda (UGA)	4		
Others			
Unknown country	11	RCB (Peru) hybrids	282
AVRDC hybrids	38	IITA hybrids	1,038
Subtotal	2,440	Subtotal	3,086
Total	5,526		

Quarantine restrictions prevent CIP from conducting an adequate characterization of all accessions collected outside Peru. Therefore, CIP is undertaking morphological characterization and preliminary evaluation of sweetpotato in collaboration with NARS in each country of origin. This in-country characterization not only facilitates the identification of duplicates in these collections but also helps to determine genotypes with desirable traits and good breeding potential. Progress has been made in characterizing sweetpotato

collections in Saint Vincent, the Dominican Republic, Jamaica, Paraguay, Argentina, Brazil, and Mexico. To date, 1,373 accessions have been characterized. Of these, 731 were found to be potential duplicates that might comprise only 169 different cultivars.

Studies of genetic diversity based on RAPD markers or DNA amplified fingerprints (DAF) showed that sweetpotato exhibits a very high degree of genetic polymorphism. Several accessions cluster together based on their geographic origin. Other accessions from South America and New Guinea also cluster together, suggesting an evolutionary relatedness. Other New Guinea sweetpotatoes are dispersed across many clusters, indicating some genetic divergence, probably caused by adaptation to isolated highland ecological conditions (Jarret and Austin, 1994; He et al., 1995; Zhang et al., 1996).

Conclusion

Although much progress has been made in collecting sweetpotato genetic resources, re-collection of closely related wild Ipomoea species is needed from areas where no living materials have been obtained by previous expeditions or when few living accessions of a given species exist in ex situ genebanks. More comprehensive genetic diversity studies are also needed to determine whether the cultivated genepool available in genebanks adequately represents the genetic diversity still in existence in farmers' fields in Latin America, Africa, and Asia.

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Section 1.3

Morphologic identification of duplicates in collections of *Ipomoea batatas*

Z. Huamán

Introduction

Clonal maintenance of sweetpotato collections requires a periodic multiplication that could be done once or twice a year, depending on the environmental conditions prevailing in the area where the collections are located. In some areas, sweetpotato field genebanks are grown during the summer and the storage roots are kept in storage rooms during the cold season. The collection is then grown from stem cuttings produced from storage roots that are planted in sprouting beds. The maintenance of a field genebank is expensive

and it is exposed to a series of diseases and pests, droughts, excessive rains, etc.

A common problem in most countries that still maintain sweetpotato collections is the gradual loss of accessions in the field genebanks. Genetic losses in field genebanks occur when curators use very small plots to grow the accessions in the collection. In many countries, the use of very small plots with few plants is an alternative to reduce maintenance costs. Many sweetpotato collections are maintained only in a single pot and many losses occur during the dry periods. Another problem is the wide variation in the degree of virus infection and the presence of different viruses in accessions in the collection. Cross contamination and virus spread, within clonal collections are unavoidable.

Another important problem is the high level of mixtures between accessions in national sweetpotato collections. Mixtures generally occur because of the lack of alleys between plots which favors the invasion of accessions with very disperse growth habit. Other mixtures can also occur when the field genebank does not have an adequate crop rotation system. Volunteer plants usually develop from storage roots that stay in the field after the harvest. Incorrect labeling of accessions is also another source of mixtures especially when labels are handwritten and slight changes in accession numbers are made (Huamán, 1996).

The morphological characterization of accessions in the collection is essential not only to have a description of each accession but also to use this information to identify duplicate accessions. These data should be recorded on plants from all accessions in the collection grown under the same environment, under the same plant density, and in the most favorable season for good plant development.

Duplicate identification

Duplicates in sweetpotato collections are samples of the same cultivar that were collected in different farms of one or more localities. The presence of many duplicates increases the cost of maintenance of these collections. It also slows down the process of evaluation for desirable traits needed in breeding.

In many countries, there are several collections maintained by universities, experiment stations or private farmers. It is recommended to organize a national sweetpotato collection by implementing the following steps:

1. To introduce samples of stems cuttings from accessions of as many existing collections as possible in one site to grow them under the same environment.

2. To group the storage roots of all accessions according to their similarities of skin color, shape and flesh color. The storage roots of all accessions should be washed to see the true skin color and then grouped according to the predominant skin color. The data on skin color are then recorded in reference to a color chart that combines color and intensity that we developed to match color diversity shown by sweetpotatoes. This color chart is available upon request. Within each group of skin color, accessions are then sorted according to the shape and then according to the flesh color obtained from transverse sections of the storage roots. In this way, all accessions with identical characteristics will have the same descriptor codes. The storage roots arranged by their similarities in skin and flesh color and shape are then stored in paper bags identified with the accession number and the sequential order. There is a chance to further refine the groups of accessions with similar storage roots on the basis of sprout color.

3. To plant these sprouts in pots to improve the groups according to leaf and vine similarities of the young plants.

4. To plant in the field stem cuttings obtained from each accession. The planting should be made maintaining the groups and sequence of accessions according to the storage root groupings and to record data on the key morphological characters of vines and leaves.

5. To verify if data from accessions that are morphologically alike are similar.

6. To process the data using software for cluster analysis to group them according to similarities of all the morphological data.

7. To organize a new planting of the collection according to the groupings obtained by cluster analysis, and to make more detailed morphological comparisons between those accessions that are morphologically alike.

Huamán (1996) points out that identifying duplicates in collections of clonally propagated crops is essential because it allows the following:

- A reduction in the number of accessions to be clonally propagated to a manageable size.
- A decrease in maintenance costs. The collection will occupy a smaller area and, consequently, there will be a reduction in expenses for labor and supplies. A reduction in these costs is extremely important, especially when funding is scarce.
- More chances for survival of all the different cultivars available in the collection. In collections with many duplicates of the same cultivar, cultivars represented by a single accession can be lost.
- Determination of the geographic distribution of each cultivar.

The identification of duplicates in sweetpotato collections depends on:

- The degree of accuracy with which the data was obtained during the morphological characterization in the field. It is recommended that the same person should record the morphological data to reduce the level of inconsistencies.

- The correct transfer of the data to a file for computer processing.

- A good validation of the data once the accessions of the collection are grouped according to their similarities and grown side by side.

Data recording on morphological characters

The time to record the data on leaf and vine characters is when the plants are totally grown. Under normal conditions, it is recommended to record the morphologic data at about 90 days from planting or 15 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 sweetpotato characters of the vines and leaves vary throughout the plant, it is necessary to choose a part of the plant considered to be 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 this 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 making comparisons between the data recorded in different years.

The morphological data of accessions of a sweetpotato 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). Looking at the figures of those descriptors that show shape variation until one is used to all the

different options also facilitates the choice of a descriptor state related to shape.

The following list of morphological descriptors has been found to provide an adequate description of sweetpotatoes and are widely used to identify duplicates in sweetpotato collections. Data are recorded using a 0 to 9 scale for key sweetpotato descriptors selected from an internationally accepted descriptor list (Huamán 1988, CIP et al. 1991).

List of descriptors considered as key characters for duplicate identification

1. Vine characters

1.1 Twining

Ability of vines to climb stems of 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 (< 4mm)
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
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 leaf outline (Figure 1), type of leaf-lobes (Figure 2); the average total number of lobes (Figure 3); and the shape of the central lobe (Figure 4) of leaves located in the middle section of the vine of 90 days old plants.

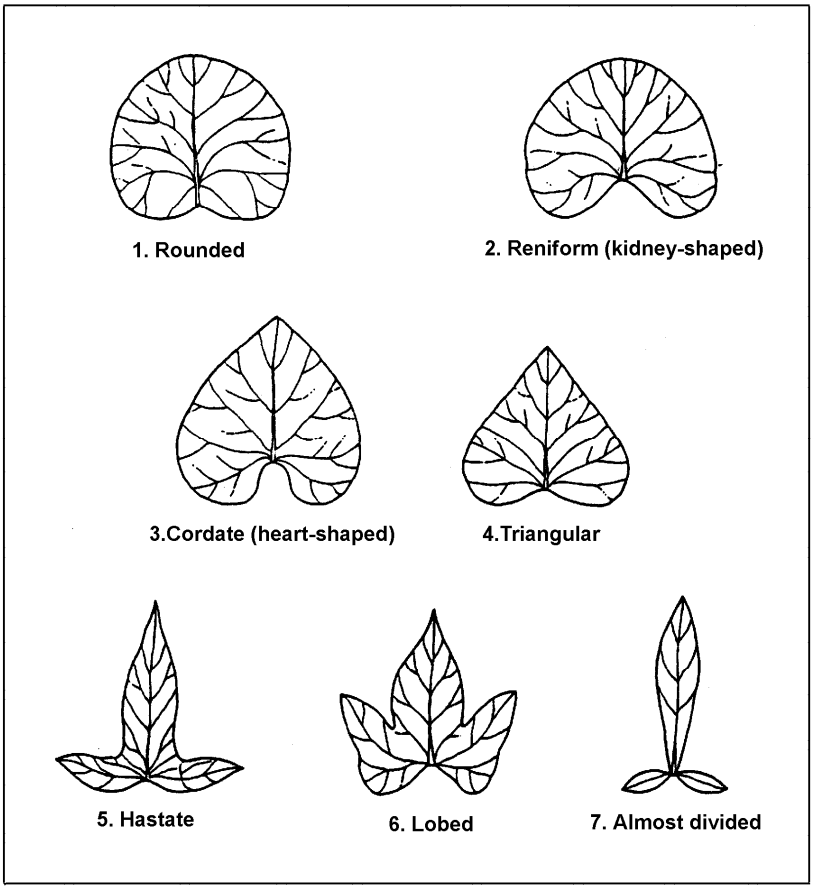

Figure 1. General leaf outline

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

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

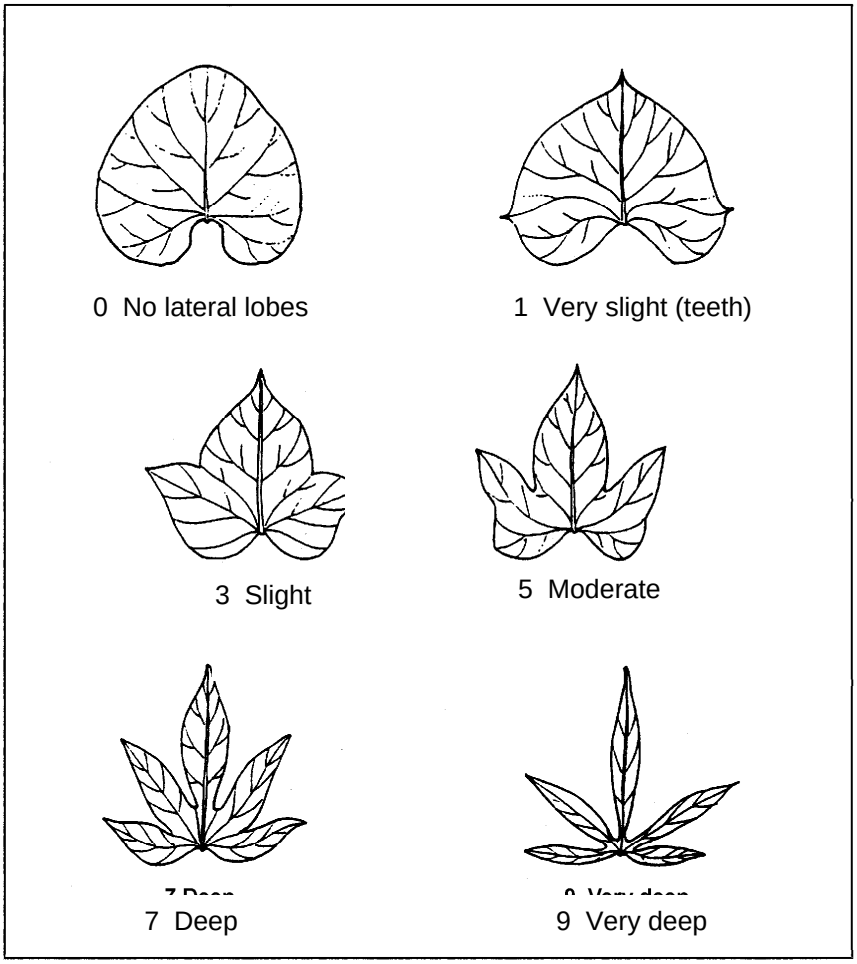

Figure 2. Type of leaf lobes

2.1.3 Number of leaf lobes

Most leaves of sweetpotatoes 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 sweetpotatoes have 1, 3, 5, 7 or 9 leafs lobes. If the leaf has no lateral lobes but shows a central tooth 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.

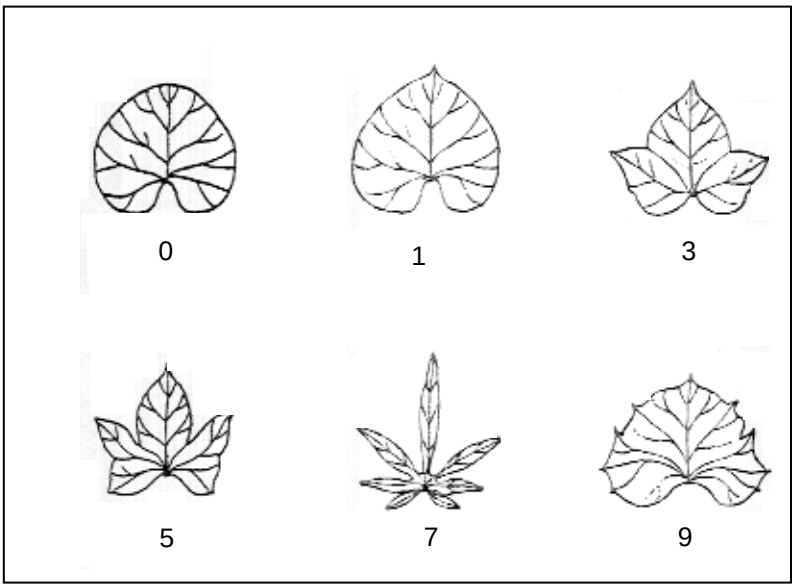

Figure 3. Number of leaf lobes

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)

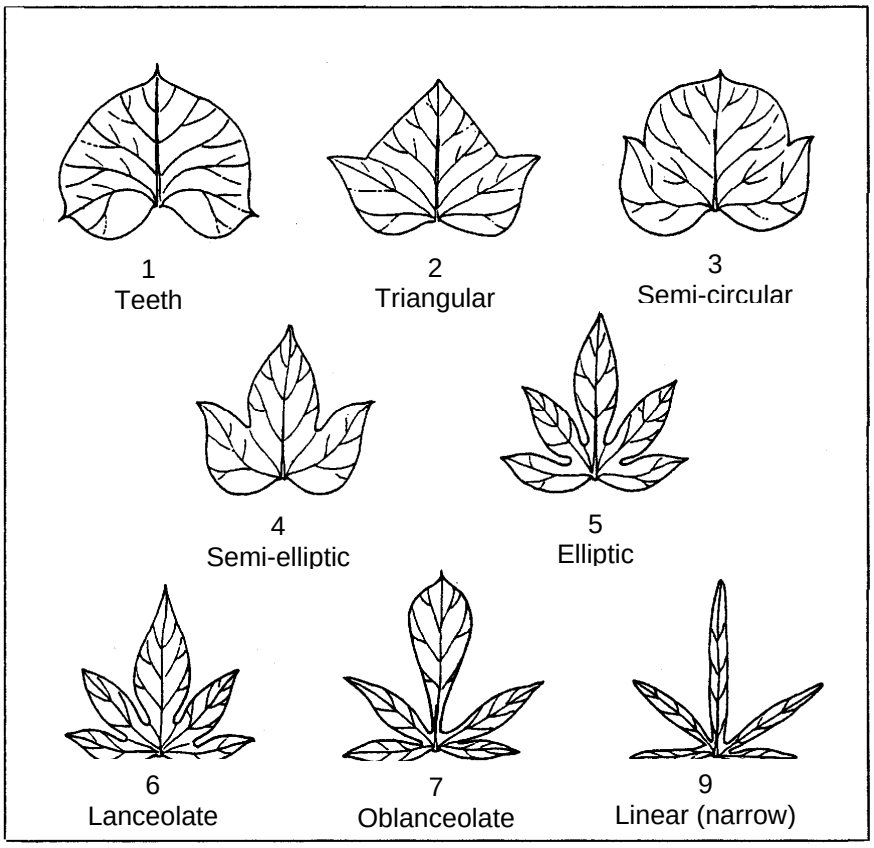

Figure 4. Shape of central leaf lobe

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 (Figure 5).

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

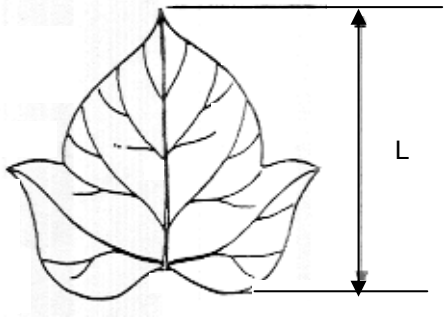

Figure 5. Mature leaf size

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 (Figure 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

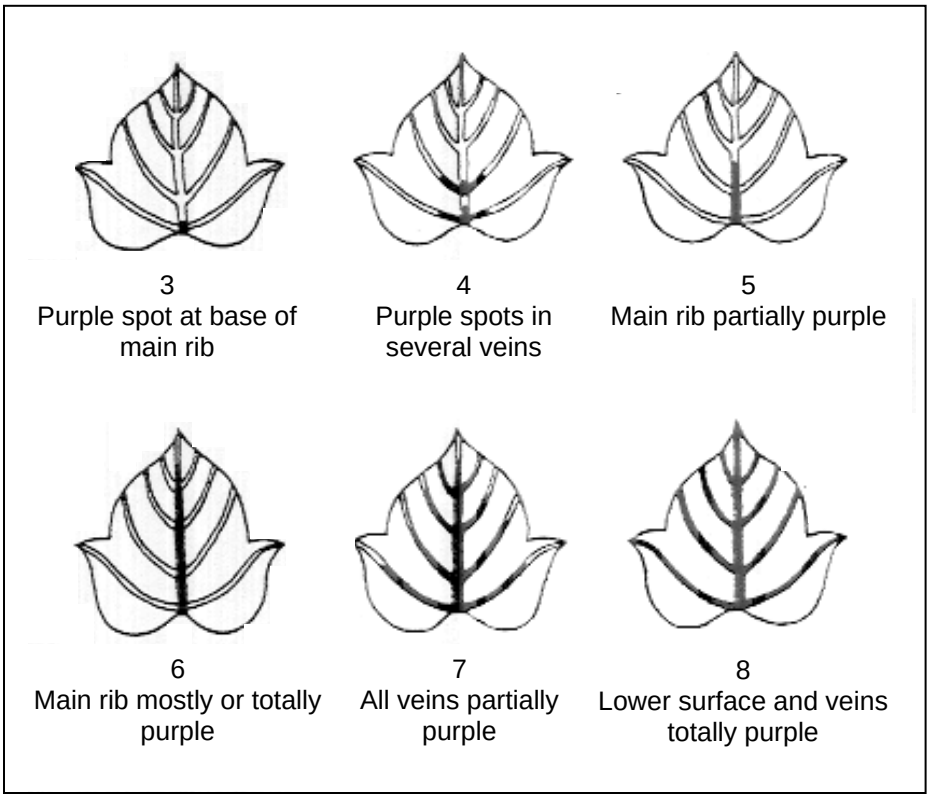

Figure 6. Abaxial leaf vein pigmentation

2.4 Foliage color

Describe the overall foliage color considering the color of fully expanded mature and immature leaves shown by several plants. The variation 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 petioles. 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 (Figure 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)

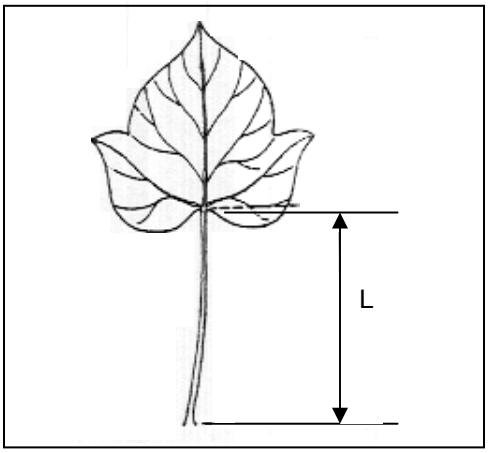

Figure 7. Petiole length

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 (Figure 8).

1 Round – almost a circular outline with a length to breadth (L/B) ration of about 1 to 1.

2 Round elliptic – a slightly circular outline with acute ends. L/B ration 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 en (i.e. opposite to the stalk of the root).

5 Ovate – inversely ovate outline. The broadest part is at the proximal end (i.e. close to the stalk of the root).

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 an 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.

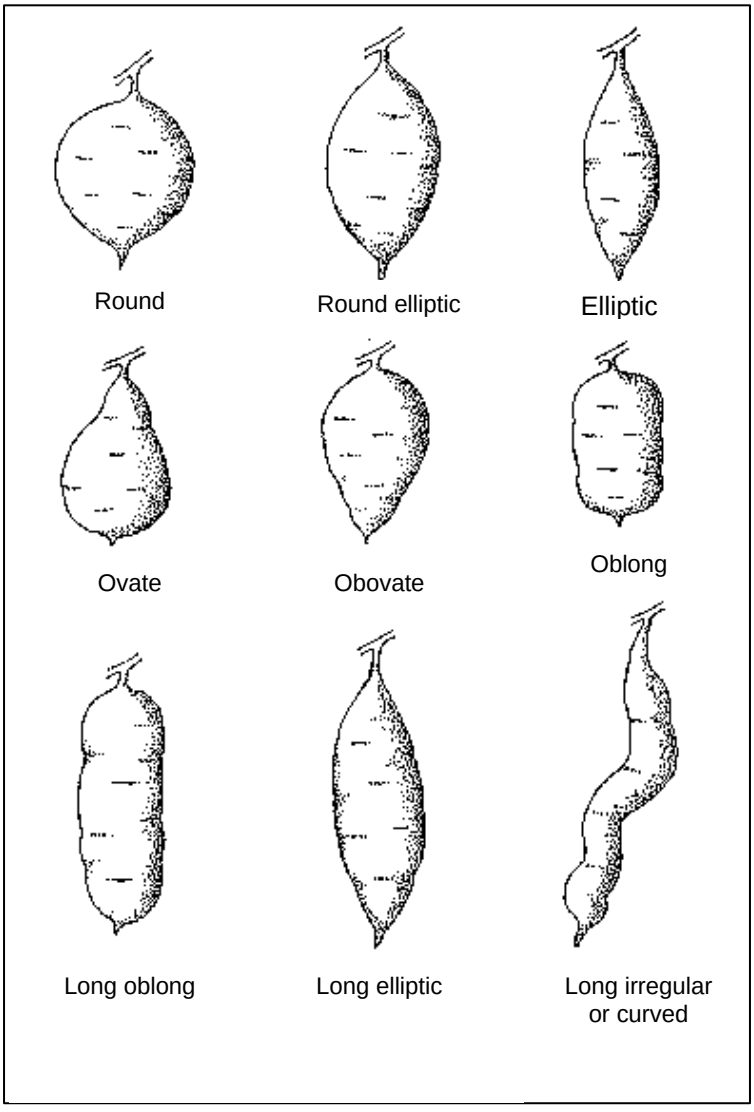

Figure 8. Storage root shape

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
9

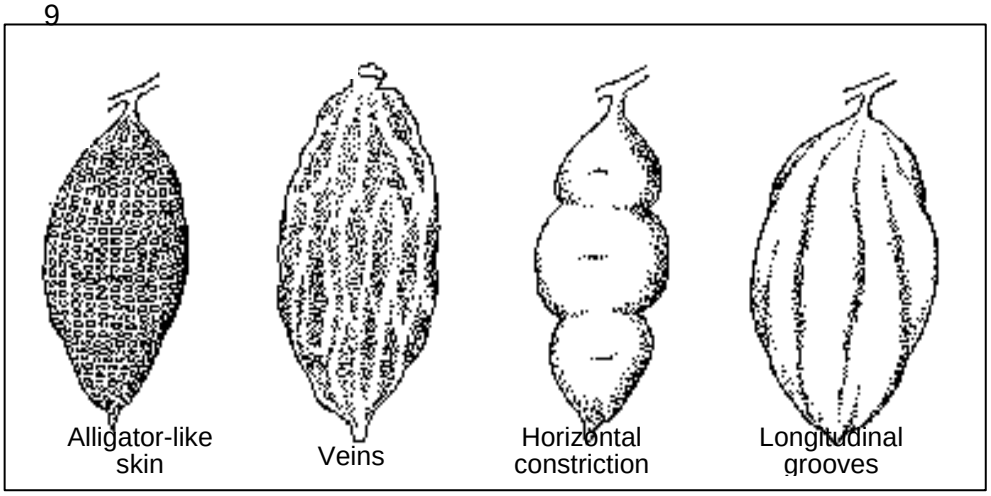

Figure 9. Storage root defects

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)
10 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 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

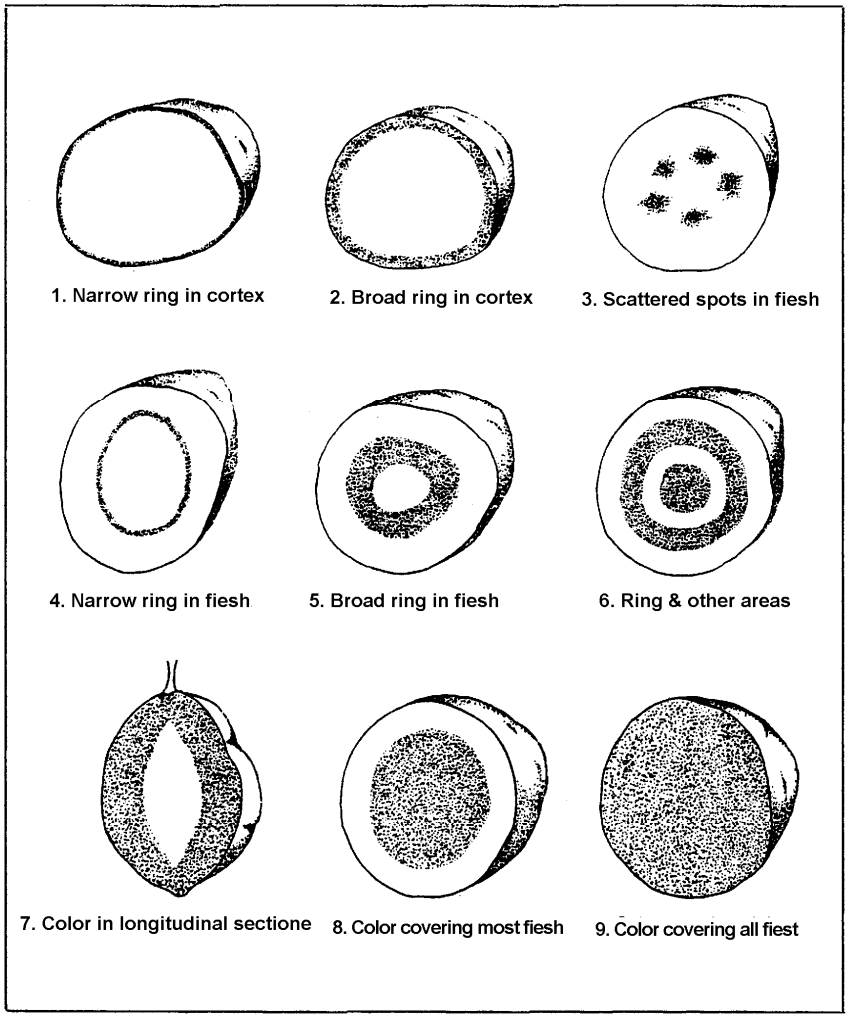

Figure 10. Distribution of secondary flesh color

3.6 Storage roots arrangement

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

1 Closed cluster
3 Open cluster
5 Disperse
7 Very disperse

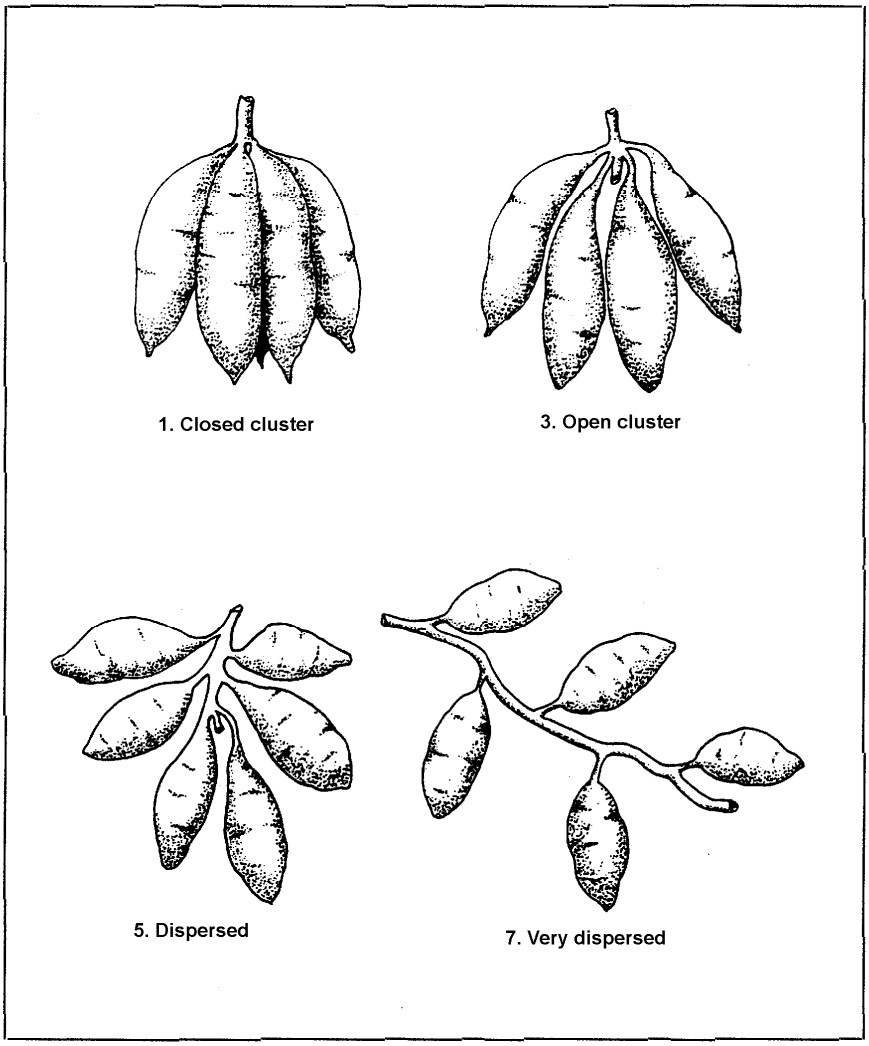

Figure 11. Storage root arrangement

Data Processing

Once the data recording has been finished in the field, they have to be organized in a computerized database using commercial software (Dbase, FoxPro, Microsoft Access, Microsoft Excel, 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.

The best groupings of morphologically similar accessions are obtained by using software for CLUSTER ANALYSIS that are commercially available in statistical packages like SAS (SAS, 1985), NTSYS-pc (Rohlf, 1992), etc. The advantage is that all accessions in one cluster share many morphological characters in common. Those accessions that are 100% similar are likely to be duplicates. In those accessions that are more than 90% similar, one needs to check if the differences in 1, 2 or 3 descriptors are real or are due to inconsistencies at the time of data recording.

Since NTSYS-pc is inexpensive software for numerical taxonomy analysis widely used by researchers, the options used to process the sweetpotato morphological data are as follows:

1. Convert your data file to a text format following the structure accepted by NTSYS-pc. Data for each descriptor should be in one column.

2. Convert the codes for predominant storage root skin color and the predominant skin color intensity shown in the color chart into sequential numbers from 1 (white-cream) to 26 (dark purple). Thus, 1=12, 2=13, 3=21, 4=22, 5=23, 6=31, 7=32, 8=33, 9=41, 10=42, 11=43, 12=51, 13=52, 14=53, 15=61, 16=62, 17=63, 18=71, 19=72, 20=73, 21=81, 22=82, 23=83, 24=91, 25=92, and 26=93.

3. In NTSYS-pc select the **Similarity** menu and choose the option **SimQual** (to determine similarities for qualitative data) and the Simple Matching coefficient. Then, select the **Clustering** menu and choose the **SAHN** option with the method UPGMA (unweighted pair-group method using an arithmetic average). Finally, select the **Graphics** menu and choose **Tree Plot** to print the dendrogram.

In the case that the researcher do not have access to a software for cluster analysis, it is possible to group the accessions of the collection according to their similarities 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, immature leaf color, 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. All accessions with the same data for those descriptors will be grouped. The only disadvantage of this type of sorting is that all accessions that are morphologically similar will be separated according to the values of the descriptors they differ. Duplicate accessions with wrong values for one or more descriptors will be totally separated from the group.
If the collection is planted on the basis of the groupings, obtained either by cluster analyses or by the sorting of the most important characters, it is easier to verify consistency of the data. It is not unusual that the same cultivar could have different data in some characters caused by inconsistencies of the evaluator or by environmental effects such as the watering, fertilization, shading, etc.

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 sweetpotato cultivar.

Since sweetpotato 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 easily is the storage root flesh color.

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

Duplicate accessions of the same cultivar will also show similarities in other characters. Among other these characters are earliness in storage root formation, yield potential, reaction to diseases, pests, and environmental stresses present in the area where the collection is grown, ability to flower under natural conditions, degree of stigma exertion, changes in the flesh color of boiled storage roots, etc. All accessions that are truly duplicates will show the same physiological reactions. However, there might be slight differences in vegetative period and yield potential because of variations in the degree of virus infection in the different accessions of the same cultivar.
All duplicate accessions of the same cultivar must be flower more or less at the same time and with the same intensity. If they do not flower, the requirements 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.

Depending on availability of facilities in the country, more sophisticated tests can be made to further verify that two or more accessions are truly duplicates. One can make comparisons of the banding patterns of total proteins and esterases extracted from storage roots, which are separated by porosity gradients and discontinuous polyacrylamide gel electrophoresis (Huamán & De la Puente, 1988). For those institutions with equipment for DNA fingerprinting, a fast duplicate verification could be obtained using RAPDs. Zhang et al. (1997) have shown that using three highly polymorphic RAPD primers will give enough accuracy for verifying morphologically identical duplicate accessions in sweetpotato. RAPD comparisons should be conducted making sure that all duplicate accessions of the same group are in the same gel. That makes the operational error negligible, because the cross-plate and cross-gel comparisons are avoided. However, RAPD is unsuitable for assaying intra-varietal variation in sweetpotato. Therefore it may not be a good tool for monitoring clonal genetic stability or detecting somaclonal mutation in sweetpotato. Those accessions of the collection that are morphologically identical and have the same DNA fingerprints are considered duplicates of the same cultivar.

Clonal Conservation of representative accessions of duplicate groups

Once the duplicates have been identified, it is recommended to transfer to in vitro culture at least one accession representing to each duplicate 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.

Conversion to true seed all those accessions that are found to be duplicates

A curator should not be afraid to loose valuable genes from accessions that are truly duplicates or of those that are morphologically identical but might have slight differences caused by mutations that can not be observed in the field genebanks. In order to preserve those genes that provide adaptation to the environment were they have been grown for many years, including adaptation to edaphoclimatic, disease and pest, and human-imposed factors (Carey, 1996), the production of geographical seed pools is recommended. This is accomplished by planting all accessions to be converted to seed in isolated plots or polycross nurseries grouped according to the eco-geographic zones where they were collected. For this the availability of passport data of accessions in the collection is of prime importance. It is also important to randomize the

accessions in such a way that identical cultivars should be separated by other different cultivars from the same geographic origin. This also should favor the presence of different self-incompatibility groups in the different cultivars, which will increase the chances for seed set.

If there are many cultivars within a geographic group, one can make sub-groups according to the Vegetative period to harvest (90, 120, 150, >150 days from planting) or organize them according to storage root characteristics of importance in the country. All open pollinated seeds should be collected from each accession aiming to have a minimum of 1500-2000 viable seeds or higher in genetically heterogeneous crops like the sweetpotato so that to have enough seed for regeneration and monitoring tests of viability (Genebank Standards, 1994). All seeds of each accession should be kept separate maintaining the accession number, duplicate group to which it belongs, and the geographic group. Dry the seeds to about 5% moisture content, place them in hermetically sealed containers and store them under sub-zero temperatures.

The accessions of a sweetpotato collection generally show a wide variation in the degree of flowering, ranging from none, scarce, to profuse. Several techniques have been developed to promote not only sweetpotato flowering but also for the fruit and seed set. These are short photoperiod, moderate temperature, limited water supply, grafting, trellises, growth regulators, over wintering, and vine girdling among others. In practice, a combination of these methods are used (See Section 2.6).
Abundant seeds have been produced in a large number of Peruvian accessions maintained at CIP with the following procedure:

- **Short day treatment**

Twelve stem cuttings are obtained from each accession in the collection to be converted to seed and taken to an area where they are planted in small pots (2 cuttings per pot). These pots are placed in beds with frames to hold a black plastic or other dark material that produces total darkness inside for the short-day treatment. In places with high night temperatures, these tunnels like structures should have a ventilation system to avoid too much heat stress to the young plants. Apical shoot tips are removed from the young plants before starting the short day treatment to stimulate the development of axillary buds. The tunnels are covered at about 4 p.m., before the workers go home, and removed the next day at about 8 a.m., when they come back to work. The plants receive this short day treatment for 1 to 2 months and then they are transplanted to the field. The best season for seed production is when temperatures are about 25^{o}C and scarce rains are expected.

The plants are then transplanted to the field in two consecutive rows containing 3 plants each spaced every 50 to 75 cm. All accessions

from the same geographical area are planted in the same field and watered frequently until plants are established and afterwards, watering is reduced to create some water stress.

- **Use of trellises**

Once the plants are of about 50 cm high, bamboo stakes are placed by each plant in the plot of each accession, crossed at the top and joined with a horizontal stake. The stems are tied with strings to the trellises as they grow. This will allow the plants to have an upward vegetative growth, receive a higher exposure to light, and will also facilitate the harvest of capsules.

- **Vine girdling**

When the plants are about 1 m high, the main vines are girdled to delay the bulking of storage roots. For this, a broad ring of the cortex of the vines is removed from the base of the main vines.

Once several accessions are producing buds, beehives should be placed in the field to favor cross-pollination between neighboring accessions. The combined effect of using these techniques to induce flowering in sweetpotatoes is the production of abundant capsules and seeds from most accessions in the collection. So far, there was no need to use grafting to promote flowering in Peruvian sweetpotatoes. However, this technique when used in seed increase of wild *Ipomoea* species gives better results when the grafted plants receive a short day treatment.

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Table 1. Data sheet for characterization of national collections of *Ipomoea batatas*

Plot	Identification number	Twining	Plant type	Vine					Mature leaf					Abaxial leaf vein pigmentation	Foliage color		Petiole		Storage root										
				Internode		Pigmentation			Shape													Skin color							
				Diamtr.	Length	Predom. color	Second Color	Tip pubesc.	Gral outline	Type of leaf lobes	Number of lobes	Shape centrl lobe	Size		Mature leaf	Inmature leaf	Pigmentation	Length	Shape	Defect	Cortex thickness	Predom. color	Intensity	Second. color	Predom. color	Second. color	Distrib. of color	Arrangement

Table 2. List of key characters for duplicate identification in *Ipomoea batatas* collections

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

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)

Vine 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)

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)

Vine Pigmentation

Predominant color
1 Green
3 Green 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

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

Vine tip pubescence
0 None
3 Sparse
5 Moderate
7 Heavy
9 Very heavy

Mature of Leaf Shape

General outline of the leaf
1 Rounded
2 Reniform
3 Cordate
4 Triangular
5 Hastate
6 Lobed
7 Almost divided

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

Number of leaf lobes*
0
1
3
5
7
9

* Excluding the two basal leaves

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)

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 or totally purple
6 Main rib mostly or totally purple
7 All veins partially purple
8 All veins mostly or partially purple
9 Lower surface and veins totally purple

Foliage Color

Mature leaf size
3 Small (<8 cm)
5 Medium (8 – 15 cm)
7 Large (16 – 25 cm)
9 Very large (>25 cm)

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

Immature 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

Petiole pigmentation
1 Green
2 Green with purple near sterm
3 Green with purple near leaf
4 Green with purple at both ends
5 Green 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

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)

Storage root shape

1 Round
2 Round elliptic
3 Elliptic
4 Ovate
5 Obovate
6 Oblong
7 Long oblong
8 Long elliptic
9 Long irregular or curved

Storage root defects

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
8 Other

Storage root cortex thickness

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

Storage Root Skin Color

Predominant color

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

Intensity

1 Pale
2 Intermediate
3 Dark

Secondary color

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

Storage Root Flesh Color

Predominant 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

Secondary color

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

Distribution of 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
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

Section 1.4

Interdisciplinary collection of *Ipomoea batatas* germplasm and associated indigenous knowledge in Anggi, Irian Jaya, Indonesia: The approach, the methods and the problems

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(Reproduced from: Collecting Plant Genetic Diversity. I. Guarino, Ramanatha Rao, V., Reid, R. (ed). IPGRI 1996.)

Introduction

Sweetpotato constitutes a primary food source in a number of areas in Indonesia, particularly amongst populations in Irian Jaya, the western half of the island of New Guinea. Whereas per capita production for the whole country is currently around 12 kg (total annual production is a little over two million metric tons), in Irian Jaya per capita production stands at around 100 kg (total annual production is about 180,000 metric tons) (Figure 1). In Irian Jaya sweetpotato is consumed as a staple mainly in the mountains, for example in the Regency of Jayawijaya, the Regency of Jayapura, the Regency of Paniai, the Regency of Manokwari, and the Regency of Sorong (Map 1). In other parts of the mountains, taro is the primary staple but in many of the coastal areas, especially the swampland to the south, sago is a more common staple. Rice is increasingly consumed in urban centers.

Whereas taro (*Colacasia* spp. and *Alocasia* spp.) and sago (*Cycas circinalis* L.) are indigenous to Asia, sweetpotato (*Ipomoea batatas*) is almost certainly of Central and South American origin (O'Brien 1972). Recent archeological research discussed by Yen (1982) suggests that it may have arrived in New Guinea from South America via Polynesia well before it was introduced in other parts of Asia by the Spanish. Since its introduction into New Guinea, it has displaced taro from many areas, and has been responsible for major shifts in social and political organization through its association with pig raising. It is currently the major staple crop. An enormous number of cultivars have been selected and conserved by New Guinean people attesting to its economic importance.

Collection and evaluation of this rich New Guinean diversity for the benefit of improved sweetpotato performance there and elsewhere has been conducted almost exclusively in Papua New Guinea (Takagi, 1988), with relatively little collection in Irian Jaya. Almost no effort has taken place in either region to systematically document local knowledge of New Guinea varieties in order to accelerate characterization and use of cultivars. This case study records the preliminary efforts of members of the Root and Tuber Crops Research Center of Cenderawasih University, Manokwari, Irian Jaya, supported by the User's Perspective With Agricultural Research and Development (UPWARD) and by the International Potato Center (CIP) to systematically collect both the wide range of sweetpotato germplasm and its associated indigenous knowledge. It attempts to document both the benefits and the pitfalls of the methods developed for this purpose. The approach and methods discussed in this paper resulted from a fieldwork-based training workshop held in Manokwari and Anggi, Irian Jaya, Indonesia from February 3-12, 1992. The substantive results of the fieldwork are reported in Sawor *et al*. 1993.

Philosophy and approach

For collecting, at least three key reasons are commonly cited: to enlarge scientific understanding of biological and evolutionary processes; to preserve the world's biodiversity for future generations in the face of severe threats to ecosystems and hence to biodiversity at the present; to make available new cultivars to farmers and new germplasm sources to plant breeders for the development of improved varieties. Curiously, most of these activities have been undertaken as if collecting is discovering. Whether wild or cultivated, plants are too often automatically treated as unknown genetic packages waiting until Science reveals their secrets. Left out of the picture are the originators of cultivated plant germplasm and the specialists of wild species, in other words, gatherers, hunters and cultivator groups the world over. Although many scientists recognize the role cultivators have played developing the present day diversity of landraces, far fewer recognize this is part of broader expertise which rural people have of their local environments and hardly anyone had yet tried systematically to incorporate this local knowledge into germplasm conservation and evaluation.

The approach taken to sweetpotato in Irian Jaya is to recognize that the assiduous cultivation of sweetpotato diversity evident there reflects a sophisticated knowledge of the crop, which has evolved along with the germplasm. This knowledge is as much a resource as the physical material it illustrates, contributing to more effective use of sweetpotato genetic materials at the present, as well as being of potentially vital importance in an uncertain future. However, like the germplasm itself, this knowledge is in danger of disappearing, as new varieties and other modern technologies are introduced and is in urgent need of conservation.

Methods

The methodology draws on a combination of conventional collecting practices, rapid, participative survey techniques (PRA) and ethnobotanical elicitation.

Team size and composition

Mobility in Irian Jaya is complicated by lack of roads in the interior (except in the central Baliem Valley region) and the consequent need to take light planes often limited to four or five passengers. Since there are no shops and very few markets, it is also necessary to take most provisions. Both these factors limit the possible size of the collecting team. In this example, the local team consisted of four people, and since the activity was also part of a training and methodology development activity, two additional resource persons took part.

From other studies of sweetpotato agriculture in Asia, we know that women are often important repositories of expertise and principal managers of the crop, and it is therefore essential to involve local women in the documentation process. The presence of women amongst the team members can help very much to ensure female input. One member of the team in Irian Jaya was female.

Interdisciplinary is a key ingredient of group composition. The essential spread of disciplines should, if possible, cover genetic resources/taxonomy interests and social sciences expertise. It is rare, however, for provincial root crop research centers to have taxonomic specialists, and in the present case two members of the team were agronomists with exposure to genetic resources issues through attendance at courses. On the social sciences side, the original plan had been to include an anthropologist from the Social Sciences Faculty in Jayapura, but this proved logistically too complicated to set up[1]. Both social scientists were trained in a broad "socioeconomics" course within the Faculty of Agriculture in Manokwari.

The distinction between multi- and inter-disciplinary needs emphasizing here: whereas "multi" implies several areas of expertise and points of view, which is good, "inter" implies the interpenetration, interdependence and sharing of those points of view, which is better. In other words, the team was not expected to establish an inflexible division of labor between plant collectors and knowledge collectors. Rather, in mixed pairs or as a group, specialists were expected to take the lead in their own area of specialization, whilst participating in all types of cultivar and data collection.

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[1] The University of Cenderawasih has two campuses. The main campus is located in the provincial capital of Jayapura. The Agriculture Faculty (and Root and Tuber Crops Research Center) is located in Manokwari, in the western part of the island. Communications between the two campuses is not always easy.

Sampling

The unit for sampling was taken as the sweetpotato plot rather than the farmer. Sample plots were chosen where possible on the basis of an apparently large number of sweetpotato cultivars growing there. It was also important to choose plots where the owner was known and could be located. There was also an attempt to spread the plots among different hamlets of the village to maximize cultivar range and possibly ecological diversity. These criteria were not always compatible, particularly the need to locate the cultivator(s) of a genetically diverse plot. This led to a slower collection process than would have occurred in a "conventional" collecting expedition.

For each cultivar found in the plot five proximal stem cuttings were taken, each having a length of approximately 50 cm. Where only one or two plants of a cultivar could be identified, fewer cuttings were taken to avoid the risk of the farmer losing the variety. Where possible we avoided duplicating cultivars collected from the same plot but no attempt was made to avoid collecting what appeared to be the same cultivars from different sample plots, simply because duplication could not be conclusively determined in the circumstances.

Our concept of "plot" turned out to correspond to a rather complicated notion in Anggi. Rights of access to particular areas of land are determined by tribal affiliation, but ownership or use of particular "gardens" (*ro*) is by household, with a number of kinship-related households often opening a new area together, but separately planting adjacent "gardens" within the area. We were interested that the concept of "sample plot" should coincide with the "garden" cultivated by a single household in order to make more specific the local knowledge elicited from the farmer. In several cases, however, the enthusiasm of farmer consultants led them to dig up cultivars planted by different households in adjacent gardens. This also complicated our ability to collect data on the precise number of cultivars each household managed.

Documentation of Information

There are at least three pools of information associated with plant germplasm collection:

- The genetic make-up of the plant
- The cultural knowledge about the plant
- The cultural, socioeconomic and ecological characterization of the plant's "context" or environment.

At the time of collection, little can be directly learnt about the first information pool, apart from the gross observable characteristics of cultivars. Simplified passport data sheets were used to register the

normal passport information such as location of specimen, assigned collection number, local name, etc., and also this basic information about each cultivar, such as root skin and flesh color, shape and a few special characteristics, such as earliness forms of utilization. Some of the simpler physical conditions of the sample plot such as slope, soil type and stoniness are also recorded on these sheets (Table 1).

The second and third information pools were handled through a topic guide sheet divided into three sections:

- the ethnobotany of *Ipomoea batatas* and individual cultivars;
- characteristics and management of the sample plot;
- broader "contextual information" about local cropping, farming and livelihood systems.

Topic guides were worked out during the pre-fieldwork planning sessions based on secondary information we had available, the field experiences of the local team members, and a reconnaissance trip we conducted in the area some months earlier. These were then amended and improved during the fieldwork itself.

Data gathering included a range of methods.

Informal interviews

Informal interviews with individual farmers were conducted during the extraction of roots of individual cultivars, to gather preliminary cultural information on the particular cultivar and on the history of the plot. This type of interviewing proved more successful for gathering data on the plot than individual cultivars, since it was found that discussion of a cultivar in isolation is often difficult and besides, there was little time to get into great detail at the same time as collecting and labeling specimens. It was therefore decided to concentrate questions and observations on a few major characteristics of the cultivar (local name, plant type, flesh and skin color, time to first harvest, etc.) and to gather information on the plot. The rhythm of collecting proved in fact being an occasional source of conflict between the disciplines. The collection of specimens and the filling out of basic information in the passport form was relatively easy and fast. Eliciting more detailed information on the plot and the cultivars was much more time-consuming, limiting the number of plots that could be sampled in a day. A second type of interviewing with individual farmers was conducted in his/her house later in the day, to go over the evaluation of the cultivars in detail.

Our experience with the individual interview was not very encouraging but this could have been partly due to the elicitation method utilized at first, involving discussions of individual cultivars in turn, rather than

comparing and contrasting cultivars. It was also partly due to the type of individuals whose plots we were first taken to and who were the first interviewers. They were the local political leaders and perhaps not necessarily the real experts on sweetpotato cultivars. Obligations to these local leaders who grant permission for collecting to take place must be honored of course, but care should be taken that they do not undermine the search for local expertise. We would propose wider testing of comparative evaluations with a bigger range of local individuals, especially involving women. On several occasions the team subdivided, and the female team member led interviews with women farmers. These worked quite well when conducted in the home, but in the field the men who were present tended to answer for the women who demurred.

Key informant interviews

Key informant interviews were used mainly to obtain contextual data and to a lesser extent to discuss the classification and other aspects of cultivars. An employee of the Kecamatan (local District Administrative Office) served as a key informant in the village of Iray. A Rural Secretary acted as a key informant for the village of Sururey. Both key informants were respected elders in the villages where they hold these posts. To help with our effort to understand local cropping practices and the farming and livelihood structures, a number of participative rural appraisal (PRA) techniques were utilized as part of these key informant interviews:

- personal biography was elicited to help understanding of the dynamics of the local communities and the significant local events, which had occurred.

- transects were drawn up of both villages to show different local use of land and resources and to highlight potential problems associated with resource degradation (Figure 1).

- social and resource maps characterize land distribution and important institutions in the sites (Figure 2).

- seasonal and yearly calendars show cropping systems and farmer management activities, and work diagrams show the division of labor among men and women (Figure 3).

- planting material flow diagrams helped to illustrate the management of continuity in plantings (Figure 4).

- n retrospect it would have been possible and perhaps more beneficial to have involved groups rather than key informants in these PRA activities.

Figure 1. Transect of the village of Iray showing the distribution of natural resources.

Type of Land	Upland	Midland	Marshland	Lake
Soil	Compact clay soil	Loam with stones	Organic	
Crops	None	Planting on the agricultural land is divided into 2 cycles Cycle I kidney bean, maize, sweet potato, pumpkin Cycle II kidney bean, maize, sweetpotato, pumpkin, shallot, garlic, onion, shallot, garlic, onion, carrot, potato Homeyards fertilized with household rubbish and planted with potato, maize, sugarcane, garlic, shallot, onion, carrot, celery, sweetpotato	Drainage canals built on the marshand to enable the planting of kidney bean, maize, sweetpotato, pumpkin, carrot, potato	
Livestock	Kaskus birds	Dogs, pigs, chickens, ducks and goats	Dogs, chickens, goats	Fish, eels
Other products	Rattan firewood, building materials			

Figure 2. Map of the village of Iray

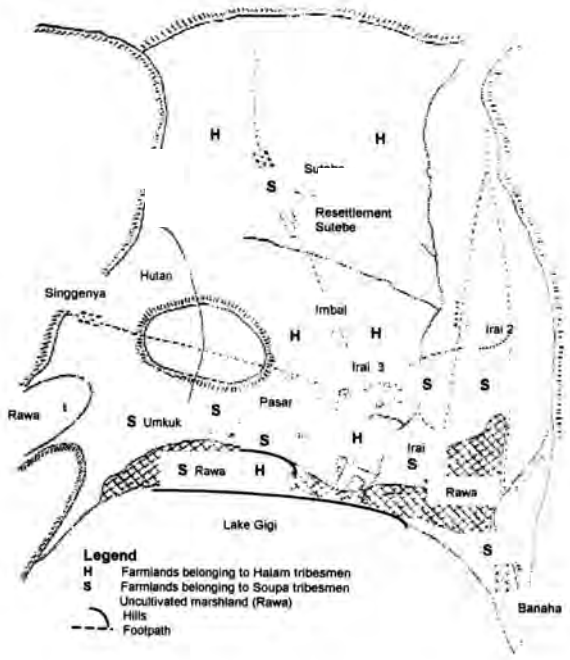


Figure 3. The cropping calendar at Iray and Sururey.

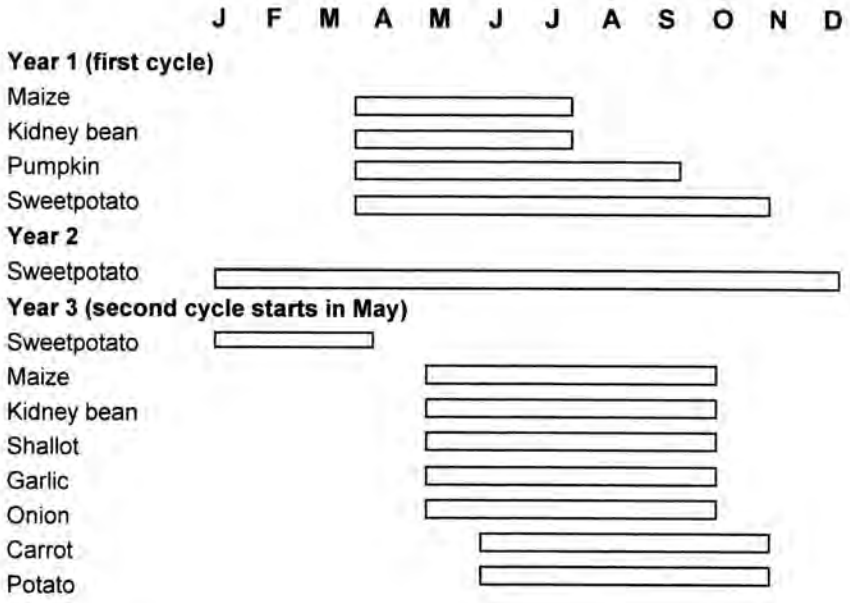
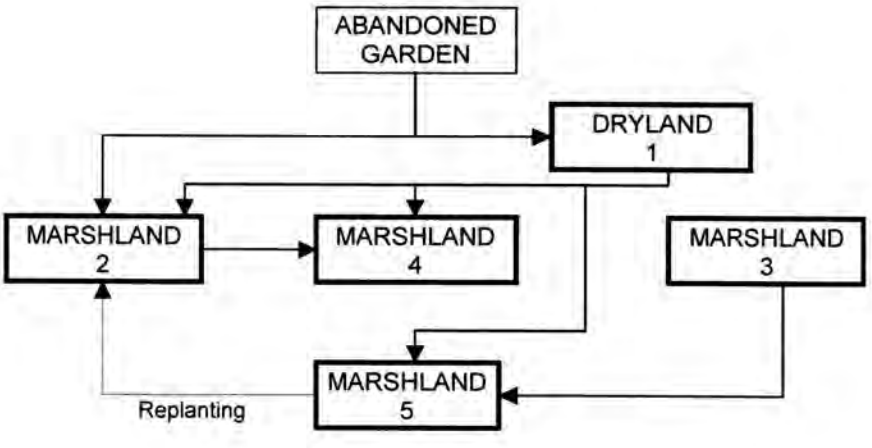


Figure 4. Planting material flow diagram for the plots owned by Markus Ahoren at Sururey. Planting material is generally obtained from old gardens. The planting material for a particular variety normally originates from more than one plot. This is so that sufficient quantity and adequate quality of material can be obtained. Maintaining a constant supply of cuttings is complex and involves the sequential planting and maintenance of plots. This diagram gives a specific example of a household "seed" supply system. Each box represents a garden plot. Numbers give the sequence in which plots are planted and the arrows show the flow of planting material. Plot 1 is the oldest existing garden. The material there came from a now abandoned plot.



Group interviews

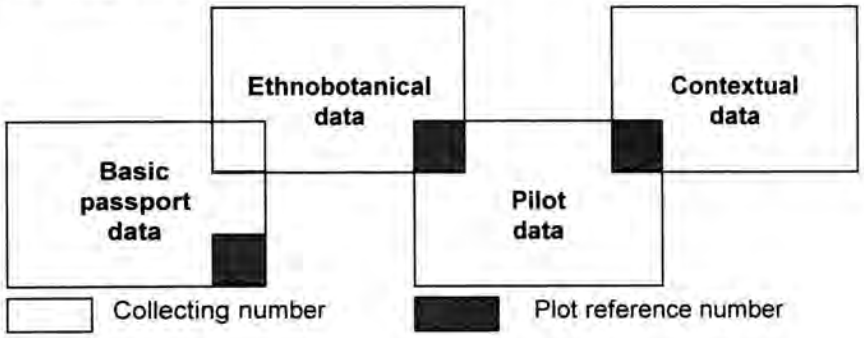
Group interviews with farmers were found to be the most successful, and were quicker to elicit salient characteristics of cultivars during the expedition. Two modalities were tested. The first was in the field plot, immediately following the harvesting of the different cultivars for collection. Both cuttings and sample roots were harvested, labeled and documented in the passport forms, and then laid out in an open area of the field. Once all the specimens were laid out in this way, the farmer, his family and any other people present in the plot (usually many!) were invited to gather around the cultivars to discuss them. Using the topic list as guide, the discussion moved through different aspects of the cultivars, from ease of establishment, rate of formation of roots, plant habits, reaction to stresses, ease of harvesting and then the whole post-harvest area of ease of cooking, different aspects of taste and storability. The field setting proved very dynamic, with interventions from both men and women. However, there were many distractions and side discussions, so that recording of opinions and evaluations proved to be quite difficult.

The second modality was to gather a group of farmers together in the evening to discuss the cultivars more leisurely in an available public or private location. This allowed more concentrated discussion in an environment where it was easier to record the observations. The site chosen by village elders in Sururey was the unoccupied house of the local forestry technician, which was also where the team stayed. This may not have been the most neutral of sites, and probably contributed to the largely male elder composition of the meeting, excluding a potentially important part of local knowledge (for more general discussion of gender concerns see below).

The aim of these group discussions was to elicit comments on cultivars which were most positively or negatively noteworthy with regard to a particular characteristic. Discussion of characteristics was iterative, the mention of one aspect - say sweetness - provoking group members to raise and discuss related aspects, for instance fibrousness. This approach has the advantage of quite quickly establishing a consensus on important characteristics possessed by certain cultivars (Appendix 2). Its disadvantage is the inability to characterize some of the more "middle of the road" cultivars which are neither "famous" nor "notorious" for any particular characteristic. Ideally, this procedure should be more "visually iterative": using a blackboard or flip chart. The assembled cultivars should be characterized by the group in the way described, so that the group can recheck and complete characteristics assigned to "extremist"

cultivars and can give more attention to "mediocre" cultivars (Figure 5).[2]

Figure 5. Organization of sweetpotato germplasm and indigenous knowledge data.



Care needs to be taken, however, that the zeal to unearth local characterization of cultivars does not end up forcing people to assign characteristics to please collectors. In general, farmer knowledge of crops seems to be strongly focused on the generic level (cf. Berlin 1992:53/54) i.e. knowledge of sweetpotato, knowledge of cassava, knowledge of taro. Within the genus, knowledge of varieties is likely to be selective and tends to be comparative, which is why the group discussions of all varieties together produced most ethnobotanical information.

An alternative approach to ascribing particular characteristics to particular cultivars is to start by eliciting the locally important characteristics themselves through open-ended interviewing, through systematic farmer evaluations in trials (Ashby 1987; Prain *et al.* 1992b) or by use of the triads test. In triads testing, farmers are asked to compare sets of three cultivars, to identify which pair is most closely related and which is the odd one out. The criterion for relating and discriminating these cultivars is left up to the farmer, and in this way salient characteristics which are used for discrimination are identified (see Sandoval 1992 for more details). This procedure is time-consuming and is therefore more suited to extended research in a single community or to "multiple visit" approaches rather than the "single visit" collecting expedition of relatively short duration undertaken in the present case. This is even more the case with trials which require a lengthy commitment by both farming families and researchers, and could well be the next stage after collection.

Data Recording and Synthesis

2 This is an adaptation of a technique used in evaluation of unfamiliar varieties used in Latin America (Prain *et al.,* 1992a). A similar argument is made in favor of the use of the triad test to identify salient evaluative characteristics (Sandoval, 1992). See below

As already mentioned, a simple passport sheet was used to record basic identification data and some additional observational data on both the cultivars and the sample plots. All team members used individual notebooks to record information coming from local people on particular cultivars, either during the collection process itself or during the evaluation sessions. Additional local information on sample plots, contextual data on farming systems and local social and political structures as well as personal observations were also recorded in these notebooks.

Every night, members of the research team discussed, debated and brainstormed over the specimens collected and the individual notes and observations recorded during the day. From these discussions a single daily record was made which was structured around the topic guide and linked to the specimens via a collection number.

After the expedition, three simple relational databases were established for basic passport data, plot data and ethnobotanical data. Ideally a fourth database including "contextual" information amenable to matrix-type storage would also be added (Figure 6). These databases were related via the accession code and a sample plot code.

The principal difficulty of data recording and data storage concerned what may be called textual or discursive information. In fact, it is rather difficult to pin down exactly how to describe this "problem" information. It is sometimes referred to as "qualitative", but this term is misleading since some particular qualities, such as root color or shape, are relatively easy to categorize and were recorded without too many problems by the team. This suggests that the issue is not whether information is qualitative or quantitative, but rather, the ease with which data can be isolated into discrete information "bits", and its susceptibility to be digitalized. Information already presented in digital form, such as dates, prices, yields, etc., are easily recorded and stored in matrix databases. Non-digital information such as color, taste or shape, which is associated with conventional classification schemes, can also be digitalized in the process of recording and thence easily stored. There will often be information loss in this process, however, and sometimes distortion. A description such as:

variety X has a blue-black color

may become recorded as:

variety X: color = purple

conforming to the preexisting classification scheme. This would defeat a major aim of the ethnobotanical elicitation work, which is to understand how local people structure and classify plants falling within the "genus" sweetpotato. The greatest problems, however,

occurred where no guide existed for a "translation" into digital form. The practice of giving part cooked and part raw roots of certain cultivars to pigs and the reasons for doing this, for example, which were described by one farmer, were either not recorded, or were recorded as:

variety X: use = pigfeed

A formulation which is readily included in a matrix database, but which loses most of the non-digital information in the original statement.

Part of the explanation for these difficulties, lies in the training of technicians and researchers. We expect to collect data, which describes in the most precise way possible the reality under investigation, so that interpretations and analyses can be made "from the outside". There is little preparation for collecting data, which includes indigenous explanation for local practices or technologies. Explanations are least amenable to translation into digital form. They require the technique of précis or summarizing, which is unfamiliar to most technically trained researchers. A great deal more effort needs therefore to be dedicated to expanding these skills, if we are to succeed in fully documenting ethnobotanical knowledge of crop genetic diversity. At the same time, data storage techniques need to go beyond the use of "memo" fields in conventional databases in order to really interface digital and textual information.

Handling of Specimens

The cuttings of each specimen were wrapped in damp newspaper to preserve them during the period of collection. The perishability of cuttings means that either, collecting expeditions need to have the ability to temporarily

- plant out the specimens during the period in the field,
- or that the expedition is short enough to allow the successful transfer of specimens to the ex situ genebank.

A third possibility would be the collection of roots rather than cuttings. The disadvantages of this are mainly the weight factor and the increased chance of spreading disease.

Conclusion

Though systematic plant collecting has a very long history in Western science, and the documentation of ethnobotanical knowledge has been for several decades a recognized sub-discipline of cultural and social anthropology, the integration of these practices is only just beginning. The case study described above was a preliminary attempt

to define a tool kit of methods, which can contribute to that integration.

The dynamics of the "interdisciplinary" relationship during collections were very variable. Though the overall rhythm of the work was determined by collection needs, the collection of multiple spheres of knowledge clearly takes more field time than labeling an accession and giving it a number. The experience so far is that the biological team members find the discussions with farmers very rewarding and see the benefits of having this information at an early stage. Of course, the boundary dividing useful and superfluous information will depend on the perspectives of different disciplines and will always be in need of negotiation. For example, biological scientists had difficulties justifying collection of data on ritual practices associated with crops and farming, even though anthropologists have clearly documented the seamless interconnectedness of "economic" and "ritual" knowledge and action in many societies (e.g. Sahlins 1972:65). As it turned out, in this first exploratory effort, ritual data were negotiated out of existence. In future, we will need to establish different intensities of data gathering for distinct spheres of knowledge. We need a minimum core set of ethnobotanical and socio-cultural data which will have to be defined, but there should also be an opportunity for follow up where important issues arise in other spheres lightly touched on.

Collecting germplasm is expensive and collecting indigenous knowledge as well considerably increases the costs. These extra costs can be justified by the increased utility of "known" material for other farmers and scientists versus the inutility of anonymous accessions. However, though the detail of documentation is constrained by the utility of what is documented, it should not be totally determined by it. Use value either for genotypes or local knowledge cannot always be known. Biological and cultural diversity need conserving for an uncertain future.

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Appendix 1. Topics and sub-topics for collecting contextual and cultivar information about sweetpotato cultivars.*

Contextual Information

(Contextual information is defined as information obtained through observation and interviewing of different informants on the functioning of the local agricultural system and especially common practices in the cultivation of sweetpotato).

Location (District, Village)
Altitude

Source of information

- group of farmers (random, local leaders, women, etc. Describe
- individual farmer (name)
- key informant (name and identity)

Population

- approximate size, type and name of ethnic group
- presence of migrants
- history of the settlement
- demographic features (migrant males, predominance of an age group, etc.)

Transept of locality with member of village if possible

- main agro-ecological zones
- associated soils
- associated crops (especially sweetpotato)
- associated livestock
- associated problems
- associated opportunities for improvements

Calendar of activities (associated with rainfall/temp if possible)

- crops (especially sweetpotato)
- livestock
- labor male
- labor female

Sweetpotato cultivation

Cultivars

- current diversity: approximate number of cultivars in the locality
- why plant many cultivars?
- compare number with the past: more or less?
- why do cultivars disappear (old bad, new good)? Is it important?
- interest in conservation of wide range of cultivars

- who plants most cultivars these days?
- important outside sources of information

Planting materials
- types used under what circumstances (be specific - tip cuttings, basal cuttings, roots combinations)
- sources of material (individual maintenance system, links with neighbors, etc.)
- form of planting (number of cuttings, how placed in ground, etc.)

Land preparation, by zone
- Planting (use of mounds, on flat, "pressing under" in garden, etc.)

Cultural practices
- Hilling up
- Weeding
- Use of organic/chemical fertilizer
- Presence of insect pests and diseases
- Use of synthetic and/or "rustic" pesticides
- Presence of other stresses (water logging, drought, rats, etc)

Ritual practices associated with sweetpotato
- at planting
- use in rituals
- use for curing
- links to women
- at harvest
- with food preparation

Uses of sweetpotato tops and roots coming from different agro-ecological zones
- estimation of percentages going to different uses.
- estimation in change in percentages during past 10, 20, 30 years
- marketing channels if any. How does it work?
- storage if any
- processing if any
- consumption

Assessment of overall role, likely changes

Sample plot for variety evaluation

Farmer details
- Name
- number of family members
- total area of farm
- type of tenancy
- most important crop

- area of SP

Plot
- agroecological zone and cropping system type
- size
- other crops, etc.)

General comments on SP crop
- production problems
- diversity of cultivars: what is the advantage?
- grouping or classification of cultivars: what are major categories?*
- has farmer encouraged diversity by preserving new types?
- have very good cultivars been lost? Why can't recover?
- marketing issues, prices, etc., of sweetpotato

Comments on cultivars planted

Accession Number/Local name(s) of variety
- known by other names elsewhere
- widely distributed over locality/other localities?

Physical characteristics (described by farmer)
- root shape and form
- root skin and flesh color
- plant type (spreading, compact, where are roots deposited?. Is it a problem?)
- plant color, texture

Vegetative Period (minimum and normal)

Productivity of roots

- number of roots
- size of roots: which important?
- performance in different agroecological zones
- performance in different soils
- performance over the last few years

Productivity of tops (abundant under different circumstances)

* This information will most effectively be elicited during the comparative evaluation of cultivars

Quality of root

- floury
- watery
- sweet
- dry
- fibrous
- other

Do family members seek it out on the plate?

Quality of the leaves

- do people/animals eat them? If so, what is important? Succulence, non-hairiness, what?
- Effects of climate
- special problems or advantages compared to other cultivars?
- Effects of insect pests
- special problems or advantages compared with other cultivars?
- Effects of diseases
- special problems or advantages compared with other cultivars?

Appendix 2. An Example of indigenous knowledge of varieties recorded in Anggi, Irian Jaya.

Classification and naming of varieties

Opportunities for collecting detailed information on the way varieties are classified and named were limited. This activity is linguistically complex and time-consuming and though important, it was decided to give preference to performance and quality data.

There are three main classes of variety based on skin color: "reds" "whites" and "yellows" and a residual category (Table B1). Information collected on an earlier visit to the area suggested that "yellows" may be more recently introduced varieties (Prain & Mok 1992) No information was collected on categorical characteristics of "reds" or "whites."

When working in unfamiliar local languages even the collection of variety names presents problems. In the completed list of names in Table B1, several have the same name and others have very closely related orthographics, which could reflect variations or mistakes in transcribing. On the other hand, different varieties sometimes have the same name, and only careful characterization can eliminate duplicates and differentiate varieties.

Naming of individual varieties occurs in various ways. The simplest describes the person making the initial introduction or the place from where the variety was brought.

For example, the Mirer variety was the one introduced by a missionary called Miller, whereas Tiom is the variety introduced from the area of Tiom in the mountainous region of Jayawijaya. Other names derive from the morphology of the plant. Informants say aug' meimar means "small leaves". Other names derive from the quality of the root, for example aug' mobatkej (transcribed augu' atkach during previous visit) means "hard", referring to the flesh quality. Some names combine both external and quality characteristics, for example bebau bob (transcribed aug' behop) meaning "white and hard".

Although some names refer to the color of either skin or flesh* or other characteristics of the storage root, farmers seem to find it easier to identify the variety of a given sweetpotato from its plant characteristics rather than its storage roots.

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* Though skin color seems to be the principle criterion for classifying some informants use flesh color. This ambiguity is found in other areas (cf. Ancheta 1992).

Indigenous characterization of varieties

Information on comparative agronomic performance was rather difficult to elicit which might be related to the almost exclusively subsistence status of the crop. There are no major pests or diseases to which varieties may differentially respond. There was an interest in "months to harvest", meaning the length of time it takes before large roots can be harvested. Five varieties were identified as maturing in four months, which is early for this altitude (Table 3). No information is available at the present time on relative yield performance, nor on differential responses to abiotic stresses

The identification of 13 varieties as "spreading" in Table B2 is more an observational datum rather than cultural knowledge. In terms of formal descriptors (CIP/AVRDC/IBPGR 1991), these should probably be described as "extremely spreading", since a preliminary ex situ evaluation of the material suggests that almost all the varieties from the area are spreading types (Mok and Schneider, 1993).

Local people consulted on the varieties showed much greater interest and willingness to differentiate varieties according to consumption characteristics. Characterizations were made according to ease of cooking and by cooked root texture and taste. As with most of these characterizations, only varieties most associated with particular qualities were mentioned, other "mediocre" varieties being simply left out. So, only four varieties were identified as really notorious for their "hardness" in cooking, while 11 varieties were noted as "soft" (Table B3). This latter characteristic needs further elucidation, since it has both positive and negative aspects. When associated with a high level of "dryness" of texture (perhaps the case with bekau ayosei or ayoseiya and bekau arpokmoi or arfokngoi which are identified as "dry" in Table B4) it may have a negative connotation since the variety would easily disintegrate in water. On the other hand, other varieties simply cook easily and quickly.

In terms of texture, local people evaluated the varieties by degree of "fibrousness" and degree of "wateriness" of roots (Table B4). Fibrousness is also a quality, which requires greater clarification to differentiate between a normally fibrous quality of the variety and the susceptibility of some varieties to become fibrous the longer they are left in the soil. These would be varieties, which are not adapted to piecemeal harvesting.

Evaluation of taste focuses on the sweetness of the variety (Table B5), with the majority regarded as "sweet", and a few identified as especially sweet or as lacking sweetness.

Section 2.1

Maintenance of sweetpotato germplasm in field genebanks

Zósimo Huamán

Introduction

Clonal maintenance of sweetpotato collections requires periodic multiplication that can be done once or twice a year, depending on the environmental conditions prevailing in the area where the collections are located. In some areas, sweetpotato field genebanks are grown during the summer and the storage roots are kept in storage rooms during the cold season. The collection is then grown from stem cuttings produced from storage roots that are planted in sprouting beds.

The maintenance of a field genebank is expensive and it is exposed to a series of diseases and pests, droughts, excessive rains, etc.

However, clonal maintenance of sweetpotato collections in the field allows us to do the following:

1. Facilitate the morphological characterization of each accession in the collection.

2. Identify duplicate accessions.

3. Determine the genetic variability of the crop in the country.

4. Determine the geographic distribution of each sweetpotato cultivar on the basis of the passport data of the duplicate accessions.

5. Have mother plants available to obtain botanical seeds, facilitate the transfer of different cultivars to in vitro culture, and have stem cuttings and storage roots available for distribution to farmers or researchers.

6. Conduct a detailed evaluation of the breeding potential present in a relatively small sample of the collection, but one that represents a broad genetic base. This facilitates the identification of genotypes with desirable attributes for use in sweetpotato breeding.

Planting the sweetpotato field genebank

The sweetpotato field genebank is usually maintained in observation plots whose dimensions vary according to the availability of land in the place where the collection is maintained. In general, each accession is planted in a plot with four rows. The distance between rows should be at least 0.9 m. The two outer rows are used as plot borders and the two middle rows are used to record characterization data of each accession. The stem cuttings for distribution are also taken from the middle rows to avoid the risk of mixtures caused by the invasion of stems from neighboring plots. Another alternative is to leave the outer rows of each plot empty. The length of the rows in the plot depends on the number of accessions in each collection and on resources available for its maintenance. Rows are usually 2 to 3 m long. It is important to leave alleys of at least 1.5 m between plots to separate them. These alleys also prevent stem invasion between plots.

The stem cuttings for propagation are obtained from the apical part of plants in good sanitary and physiological condition. Apical stem cuttings generally produce the highest yields. The stem cuttings should be 30 to 40 cm long, containing only the apical shoot and with all lateral leaves removed. Once all stem cuttings of an accession are obtained, workers should wash their hands with a solution containing soap and a disinfectant with bactericide and fungicide properties. The cutting blades or knives should also be disinfected with fire. These sanitary practices will reduce contamination by viruses and other pathogens from one accession to another. The stem cuttings of each

accession are finally tied with a string and labeled with the accession number. All stem packages are collected according to a designated order for the new planting.

It is very important to be careful about maintaining the correct identification of the stem cuttings of each accession in the collection. The labels should have the sequential number of the observation plot where the cuttings will be planted, their identification number in the collection, and the number of the plot where the previous planting was done. In general, good results are obtained by tying the stems with wide masking tape that resists soaking when the stems are disinfected in an insecticide solution. The identification numbers are written on the masking tape with a waterproof felt-tip pen. The stem cuttings are then disinfected with a 1.5 per 1000 solution of the insecticide Furadan (carbofuran) and a 1.5 per 1000 fungicide solution of Tecto 60 (thiabendazole).

At planting time, the soil should have good moisture to favor fast rooting of the stem cuttings. It is a good practice to mark the new field before planting in order to have uniform plot size, and clearly defined alleys for each accession. All stem cuttings are distributed on the plots according to the new planting order marked on the labels. It is important to have an assistant verify the sequence of planting to make sure that each accession is planted in its corresponding plot according to the field book.

At the time of planting, the stem cuttings are placed on one side of the furrow. The distance between stems is about 30 cm. All stem cuttings are then covered with soil up to the apical shoots. The alleys are also covered to facilitate water movement.

After 10-15 days of planting, each plot should be inspected to determine whether any accessions need re-planting. Otherwise, those accessions could be lost.

Harvesting the sweetpotato field genebank

The storage roots are harvested once the stems have been cut and removed from the field. Harvesting can be done manually, using tools like forks, picks, shovels, etc., to open the furrow and remove the roots. In loose or sandy soils, the furrow can also be opened using a plow or mechanical harvester drawn by a tractor. In this case, it is important that the tractor go slowly to allow workers to avoid mixtures of storage roots between adjacent plots. For this reason, if the collection is to be harvested mechanically, alleys should be at least 1.5 m wide at planting time.

The storage roots are then separated from their peduncles, which attach them to the stems, and selected by their size and sanitary condition. Those storage roots that will be kept in the storage room should be free of damage caused by insects and nematodes, and any other visible symptoms of diseases. The selected storage roots are

then placed in meshed sacks, or boxes made of wood, or plastic, which are normally used to store roots or tubers. The correct labeling of accessions is also important here. Each container should have a duplicated label with the identification number, both inside and outside. If the labels used in the field are waterproof, they are generally in a good enough condition to be used in the storage room.

Once the storage roots for the maintenance of the collection have been selected, other storage root samples are taken for other purposes such as evaluations of culinary quality, determination of dry matter content, or reaction to diseases and pests.

Disinfecting storage roots

At harvest, there are many opportunities to cause damage to stems and storage roots. Damages take place when cutting stems before harvest, when separating the stems from the storage roots during harvest, and when using tools like plows or chains. Any type of wounds, scraping, and peeling can affect the storage roots. These wounds allow fungi and bacteria to enter the plant, and can cause fast rotting in sweetpotato. It is a good practice to leave newly harvested sweetpotatoes in the sun in order to dry the latex that is produced in the wounds.

Bruises and blows should be avoided to reduce damages to sweetpotatoes. It is, therefore, a good practice to put sweetpotatoes in plastic or wooden cases that allow air circulation and make the disinfecting, drying, curing, and storing in refrigerated chambers easier, without changing the container.

The storage roots should be disinfected as soon as possible using an insecticide and fungicide solution. Greater care should be taken when the field is infected with *Diplodia gossypina, Fusarium solani,* and *Rhizopus stolonifer*. In these cases, it is recommended to use, for example, Furadan, (carbofuran), at 1.5 per 1000, Tecto 60 (thiabendazole), at 1.5 per 1000 and Botran (dichloro-4-nitroaniline) at 2.5 per 1000. Otherwise, disinfecting can be done using only Sodium Hypochlorite (used in laundry), in a 5% solution of the commercial product. In both cases, disinfecting is done, by soaking the meshes or containers with the storage roots for 10 minutes, in a recipient containing the disinfectant solution.

Curing sweetpotato roots for storage

Sweetpotato storage roots are cured by placing them in a high-temperature chamber (25-30ºC) with a high relative humidity (85-90%) for 4-7 days in order to heal all wounds. The curing period should not be too long, since it can cause an excessive sprouting in storage roots, reduce their size and their life in storage.

A curing chamber can be adapted in a closed room, with a heater controlled by a thermostat, and with an appliance to evaporate water and keep relative humidity high. If harvesting is carried out in a high-temperature season, curing can be done by covering the cases containing sweetpotatoes with wet bags, and then covering all the cases with plastic in order to increase the temperature. As there is no temperature and humidity control, it is necessary to rotate the location of the cases, so that the ones on top go to the bottom and vice versa.

Storing sweetpotato storage roots

Once the sweetpotatoes have been cured, they should be stored in storage chambers under a controlled temperature of 12ºC, and a high relative humidity, for prolonged conservation of the storage roots. Temperatures under 12ºC damage storage roots, cause inner discoloration, increase susceptibility to rot, and reduce sprouting capacity.

During curing, storage roots lose about 5% of their weight. This loss continues during storage, but at a slower rate. Storage roots that have been properly cured and stored lose approximately 15% of their weight during 5 months of storage.

During storage, periodic evaluations must be carried out to determine the condition of the storage roots and to remove the ones with moist or dry rot. This is very important since a sweetpotato collection has genotypes with different susceptibility reactions to diseases.

In some sweetpotato-producing areas with low winter temperatures, sweetpotatoes are usually left on the ground. The low temperatures kill the upper part of the plant, but do not cause serious damage to the storage roots, which will sprout again when the temperature rises.

Problems and solutions in maintaining sweetpotato field genebanks

1. Identification of duplicates in national collections of *Ipomoea batatas*

One of the inevitable consequences of collecting clonally propagated crops is the presence of many duplicates of the same cultivar within the collection. This is because the same cultivar can be found over extensive geographic areas, although it may have different common names. The identification of duplicates in the sweetpotato collection maintained at CIP has reduced to about one third the number of Peruvian accessions that are clonally maintained in the field.

Identifying duplicates in collections of clonally propagated crops is therefore essential because it permits the following:

a. A reduction in the number of accessions to be clonally propagated to manageable size.

b. A decrease in maintenance costs. The collection will occupy a smaller area and, consequently, there will be a reduction in expenses for labor and supplies. A reduction in these costs is extremely important, especially when funding is scarce.

c. More chances for survival of all the different cultivars available in the collection. In collections without duplicate elimination, those cultivars represented by a single accession have greater chances to be lost than those cultivars with many duplicates.

d. Determination of the geographic distribution of each cultivar.

2. Virus infections and their spread within collections

Collections of clonally propagated crops are gathered from plants obtained in farmers' fields as well as from field genebanks maintained by several institutions. In general, there is a wide variation in the degree of virus infection and the presence of different viruses in those collections. Cross contamination and virus spread within clonal collections, are unavoidable. For example, many potato accessions are highly susceptible to sweetpotato feathery mottle virus (SPFMV).

Among the practices to reduce genetic losses caused by virus infection are the annual elimination of the most virus infected plants of each accession, and second, the transfer of the most infected accessions to in vitro culture.

This problem is overcome by implementing virus eradication and maintenance of clean materials in in-vitro culture. The virus eradication process consists of a thermotherapy treatment of in vitro plantlets, followed by meristem culture of the most vigorous plantlets and serological tests for the most important viruses.

3. Mixtures of vegetative propagation materials among accessions

Mixtures frequently occur among planting materials of accessions during planting or harvesting. It is essential to train workers to handle genebank materials carefully to minimize this problem.

Other mixtures can also occur, when the field genebank does not have an appropriate crop rotation system, and material is planted again in the same fields. Plants usually develop from storage roots remaining in the field after harvest. It is important to promote the development of unwanted planted by watering the field where the collection will be planted well ahead of time. This will promote the development of volunteer plants, which can be removed before the final preparation of the soil where the field genebank will be located.

In sweetpotato collections, the chances are high for mixtures arising from the invasion of vines from accessions with a dispersed growth habit. Mixtures are generally more common when the collection is planted in a single row per accession, without any free space between accessions. This problem is solved, by planting 3 or 4 rows of at least 5 plants per accession. It is also important to leave alleys between blocks of plots, and to accommodate the vines within the corresponding plot.

Another problem that causes mixtures in the collection is the incorrect labeling of accessions. This occurs especially when labels are handwritten and slight changes in accession numbers are made. The use of more than one identification number (at least the plot number and the accession number), the availability of the field layout showing the sequence of planting, and the printing of labels from computer files usually minimize this problem.

4. Non-adaptation of accessions from the collection at the site where the field collection is located

Field genebanks of clonally propagated crops generally concentrate cultivars from different ecogeographic origins in one place. In some cases, the new environmental conditions may be totally inadequate or even adverse for the development of some cultivars. Thus, sweetpotato cultivars, adapted to relatively high altitudes, sometimes do not produce storage roots under very tropical conditions. Therefore, the selection of the site where the field genebank will be located is very important. A number of accessions could be lost because of a lack of adaptation. If an institution has facilities in different agroecologies, it can split the collection according to ecogeographic origin and plant accessions in the most favorable location. However, this will represent an increase in travel costs. The other alternative is to select those accessions with adaptation difficulties and maintain them in in-vitro culture.

5. Different reactions to diseases and pests in the environment where the field genebank is maintained

Accessions within collections show different degrees of susceptibility to diseases and pests present in the soil or in the environment where the field genebank is grown. Because it is important to obtain healthy storage roots for the next planting season, an effort should be made to identify the most susceptible accessions. If the problem is soil-borne diseases or pests, the most susceptible accessions should be planted in separate plots treated with soil fumigants. The separate plots could also be sprayed more frequently with preventive pesticides, when the problems are located in the foliage or stems of the plants. A combination of soil fumigation and preventive application of pesticides is often needed.

After harvest, storage roots need to be disinfected with pesticides to prevent storage losses. In some environments, some pests can force

early harvests. For example, some sweetpotato collections are grown in fields where sweetpotato weevil is a major problem. Besides planting the collection in the wet season (or using sprinkler irrigation in the dry season) and using other integrated pest management practices to reduce weevil populations, the only way to avoid serious damage to storage roots is to harvest as early as possible. The consequence is that no storage roots will be obtained from late-maturing accessions.

6. Variation in plant vegetative period and rest period of storage roots

Sweetpotato collections show a great deal of variation in vegetative period from planting to harvest. If all accessions are harvested at the same time, there will be remarkable differences in size, degree of maturity, and insect damage to underground parts. For example, when the sweetpotato collection maintained at CIP is grown under the most favorable conditions in the summer, about 18% of the accessions do not produce storage roots at harvest 150 days after planting. Therefore, it is important to group accessions according to vegetative period and plant them in such a way as to favor sequential harvests.

Sweetpotato storage roots in the collection also show differences in degree of sprouting when placed in sprouting beds after several months in storage at 12°C; they also have different rates of vine production. Some cultivars do not sprout. Knowledge of these characteristics is essential to avoid genetic losses.

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Section 2.2

Management of sweetpotato plant materials in quarantine greenhouse

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Collecting activities allow us to obtain new samples that could be introduced into the collections. It is important, therefore, to be careful in handling these materials when they arrive to the place where the collection is maintained.

Sweetpotato germplasm collectors should consider the following precautions to reduce as much as possible the introduction of new diseases or pests to the place where the collection is maintained:

- Take vegetative samples from plants that look healthy.
- Emphasize the collection of sexual seed of wild species.
- Collect stem cuttings of cultivars and, insofar as possible, make transfers to in vitro culture at the collection site.
- Avoid collecting storage roots as much as possible.

Every introduction of new materials should be done according to the rules established in each country. An introduction of new materials usually requires the following documents:

1. Phytosanitary certificate issued by the exporting country.
2. Permit of importation of the target country.
3. List of the accessions sent and their passport data from the place where they were collected.

Quarantine inspection

All materials from new collections should be inspected in a quarantine greenhouse, where all the necessary precautions should be taken to avoid the entry of new pests or diseases into the area where the collection is maintained.

If all the plant material is collected in vitro, the risk of introducing pathogens or pests will be reduced. However, in most cases, collectors obtain stem cuttings, storage roots, or seeds.

Plant materials obtained by collectors usually arrive in a container that should be opened inside the quarantine greenhouse and should be inspected by an entomologist and a phytopathologist. This inspection should be carried out as soon as possible to avoid the deterioration of the collected materials and to avoid the involuntary escape of any pathogen that could come with them.

Introduction of stem cuttings

After the quarantine inspection, the stem cuttings should be disinfected preventively by soaking them in a solution containing Furadan (2,3-dihydro-2,2-dimethyl-7-benzofuramyl methylcarbamate), Benlate (methyl 1-(butylcarbamoyl) benzimidazol-2-ylcarbamate), and Morestan (6-methyl-1,3-dithiolo[4,5-6]quinoxalin-2-one), all at 2%, for 5 minutes.

Once the stem cuttings are disinfected, put them in wide-spout containers with clean water (for example, milk bottles) and keep them

there until the axillary buds develop into lateral sprouts. During this period, you must maintain the level of water in the bottles, or renew the water totally if necessary.

When the lateral sprouts reach 10 to 15 cm in height, cut them with a disinfected surgical blade, and plant them in small containers that have a substrate with moss, soil, and sand. All the vegetative parts of the stem cuttings originally collected should be burned.

Once the plants are established and start their vegetative development, transplant them to bigger containers that have approximately 400 grams of substrate composed of 2 parts moss, 1 part agricultural soil, and 1/2 part washed sand.

These plants should be inspected frequently by specialists to eliminate those with symptoms of unknown diseases.

Introduction of storage roots

The storage roots have the potential high risk of transporting nematodes, insects, and many other pathogens. Therefore, the quarantine inspection should be more rigorous.

Those storage roots that look healthy should be disinfected by immersion in a solution with Morestan and Tecto 60, both at 2%, for approximately 10 minutes. The storage roots should be dried at room temperature and diffused light inside the quarantine greenhouse.

The development of sprouts in the storage roots is induced under environmental temperature above 20°C for about 15 days and planting them in a sprouting bed installed inside the quarantine greenhouse. The substrate of these beds consists of river sand that is washed in running water and then disinfected for 4 hours with steam at 100°C. The washing of the sand makes it possible to eliminate salts that could cause phytotoxicity in the plants.

Once sprouts of 10 to 15 cm of height are obtained, cut them with a surgical blade and plant them following the same process as the one explained for the stem cuttings.

You should burn all residues of storage roots, fibrous roots, etc., and sterilize with steam at high temperature the substrate used for the sprouting of these storage roots.

Introduction of botanical seed

Quarantine specialists should also inspect the botanical seed obtained by collectors. All those seeds that have insect exit holes should be incinerated. Those seeds that are apparently healthy

should be treated immediately with an insecticide with fumigating properties, such as Vapona (DDVP). This treatment consists in placing the seeds in Petri dishes with approximately 2 cm^2 of Vapona for about 30 days. Germplasm collectors should treat the seed with Vapona as soon as the capsules are collected.

Vapona [DDVP (ANSI), dichlorvos (ISO, BSI)] is composed of 2,2-dichlorovinyl dimethyl phosphate or 2,2-dichloroethenyl dimethyl phosphate. As a tablet, it gasifies in the environment. It is used to control weevils in grain storage.

Introduction of in-vitro micro-stem cuttings

In-vitro plantlets are also inspected, especially to establish whether contaminated by bacteria or fungi. Those plantlets that are free of contamination are removed from the test tubes by means of a disinfected clamp, and introduce the plantlet root into a hole made in the substrate of pots containing moss, or any other source of organic matter. The plantlets should not be touched with your hands. It is important to disinfect the clamp in alcohol and sterilize it over a burner before transplanting a new plantlet.

Maintenance of sweetpotato accessions in a greenhouse

In addition to the maintenance of all the accessions introduced into the collection in a quarantine greenhouse, it is usually necessary to keep some accessions of the sweetpotato collection in a greenhouse to avoid their loss. This is necessary especially when some accessions do not produce storage roots at harvest.

Always disinfect your hands and the instruments used to cut the stem cuttings of a new accession. Workers should therefore wash their hands with soapy water, quaternary ammonium (fungicide and bactericide) at 2 per 1000, and calcium hypochlorite (bactericide) at 2 per 1000.

We recommend using stem cuttings obtained from the apical part of the stem. Plant these stem cuttings in containers that have a substrate with 2 parts of moss, 1 part agricultural soil, and 1/2 part washed sand.

You can use a 12 N-12 P_2O_5-12 K_2O fertilizer in a dose of 5 g per liter of water to maintain soil fertility. Apply 300 ml of this solution in a container of 20 cm diameter having approximately 2 kg of substrate.

The most commonly used pesticides in plants that grow in the greenhouse are the following:

Common name	Chemical Name	Controls	Dose
Acarin	Dicofol	Mites	1.5/1,000
Pirimor	Pirimicarb	Aphids	1.5/1,000
Tecto 60	Thiabendazole	Fungi	1.5/1,000
Benlate	Benomyl	Fungi	1.5/1,000
Furadan	Carbofuran	Whitefly	1.5/1,000

Section 2.3

Techniques for rapid multiplication of sweetpotato planting materials

Z. Huamán, J.L. Marca and C. Aguilar

Introduction

The techniques for rapid multiplication of sweetpotato, that are used to obtain large numbers of stem cuttings in a short period of time, include the propagation from in vitro plantlets, the use of micro-cuttings with 1-2 nodes, the production of mini-storage roots, and the sprouting of storage roots. The production of genetic materials of the highest sanitary quality can be made from in vitro plantlets free of viruses, which are propagated in greenhouses or other constructions that prevent the presence of insects or other virus vectors, and that

isolate the plants from other contamination sources. These materials of high sanitary quality are used for the distribution of genetic material to users. The mini storage roots are less perishable than in vitro plantlets, which secures their reception by the users in good conditions. If these plants are produced under quarantine conditions, the storage roots harvested from them could be induced to sprout to produce more planting materials.

These rapid multiplication techniques require manpower and the availability of certain equipment and greenhouses that are insect proof, equipment to disinfect substrate, etc. However, these installations can be built with materials that are available locally.

Soil substrate for rapid multiplication of sweetpotato plants

The substrate for the beds that has shown good results to propagate sweetpotatoes contains 1 part of soil, 2 of organic matters (fine peat moss, compost, etc.), and 0.5 parts of sand. Another substrate that is frequently used consists of 2 parts of soil with abundant organic matter and 1 part of fine peat moss. The soil in this mixture should be sieved to make it uniform and to eliminate the large lumps.

The substrate should be disinfected. The cheapest method in tropical countries is to use solar energy for 2-4 weeks depending on the environmental temperature. For this treatment the beds are covered with black plastic to increase the temperature. Soil disinfection could also be made using water steam at 70-80^{o}C for 30 minutes or chemical fumigants like Basamid (Dazomet) at 40-50 g per m^{2}, Methyl Bromide (Bromo methane) at 1-2 pound per m^{3}, etc.

Transfer of plantlets from in vitro culture to propagation beds

A detailed description for the transfer of in vitro plantlets that are in a culture media within test tubes, magentas or other containers is provided in Section 2.5. All pots, beds, tables, containers for the substrate, and tools from the greenhouse should be disinfected to maintain the working area totally aseptic. The tools are disinfected by immersion in a solution containing 10 g of soap per liter of water and a solution of 10% calcium hypochlorite.

Before transplanting the in vitro plantlets to the substrate in the bed, 3-5 cm deep holes are made with a bamboo straw or a pencil. All plantlets are carefully extracted on a flat container using twisters or small forceps. Each plantlet should be carefully separated to avoid damage, excess of agar attached to the roots and stems washed, and then transplanted to 20-25 cm pots or to beds with a density of 50-100 plantlets per square meter. It is important to maintain the hands

in aseptic conditions at the transplanting time. Once the transplant is made, press the soil around the plantlet to favor a good contact between the soil and the roots. Immediately after finishing the transplanting of all plantlets, apply light watering to the bed and in the next days water them lightly and frequently.

It is important to disinfect the twisters or small forceps in alcohol and over a flame of an alcohol burner before using a new test tube or magenta containing plantlets of the same or different accession.

During the first 4-5 days, the greenhouse should be shaded and kept fresh to avoid dehydration and plant death, while the plantlets become adapted to its new environment.

Rapid multiplication from micro-cuttings of sweetpotato stems

This technique is used to increase very rapidly the number of plants. For this, mother plants with stems with 8-10 nodes are pruned to eliminate the apical shoots, which will promote the growth of the axillary buds. After a few days the axillary buds will be vigorously growing and it is time to cut micro-cuttings with 1-2 nodes with their respective leaves. The micro-cuttings are obtained using a scalpel over the stems placed on a flat surface. It is important to maintain very aseptic conditions both on the worker's hands and tools used, by disinfecting them with a solution containing 10 g of soap per liter of water. The scalpel is disinfected by immersion in alcohol.

The micro cuttings obtained are transplanted to beds with a density of 100 micro-cuttings per m^2. It is important to water the beds during the first days to avoid losses by dehydration. After 7-8 days the cuttings will have abundant fibrous roots and can be transplanted to the field (Burba *et al.*, 1987).

From one single in vitro plantlet, this technique can produce 10-15 rooted stem cuttings in approximately 50-60 days. It is possible to make 3-5 harvests of micro-cuttings with intervals of 10-15 days.

Production of sweetpotato mini-storage roots

The production of mini-storage roots from in vitro plantlets or micro-cuttings planted in beds within greenhouses is a very useful alternative for the distribution of genetic material to users. The shipment of either in vitro plantlets or stem cuttings, generally causes many losses in transit due to the perishability of this type of material (Centerpoint, 1984). However, mini-storage roots can be conserved from 10-12 months in storage at 20-24^{o}C and 75% relative humidity.

After 6-8 days from planting in vitro plantlets or micro cuttings in pots of about 25 cm in diameter, fertilizer is applied at a rate of 50 ml of a solution containing 5 g NPK (in a formula 20-20-20) per liter of water to each pot.

Once the plants develop 10-15 leaves, all leaves along the stems are cut at the base of the petioles. These petioles are planted in a substrate of river sand with particles 0.5 to 2 mm in diameter, previously washed and disinfected with sodium hypochlorite at 0.5%. The petioles of about 5-6 cm long are placed in holes of 0.5-1 cm diameter (Martin, 1982). About 1/3 of the petioles should be inside the substrate and then to secure a good standing, the sand around the petiole is pressed to favor a good contact between petiole and the soil. In the first days after planting, it is important to maintain the greenhouse shaded and fresh to avoid dehydration and death of petioles.

Immediately after the planting of petioles in the bed, a light watering should be applied. In the next days, watering will be light and frequent. It is important not to apply any kind of supplementary nutrients to the water to avoid expanding the period of mini-storage root production. The most favorable temperature for the best growth and production of mini-storage roots is 20-24^{o}C.

After 8-10 days from planting, the petioles will form fibrous roots and after 75-90 days will produce 1-3 mini-storage roots of about 2 to 5 g per petiole. The use of growth regulators like Benomyl (benzil amino purina) at 50 ppm, increases the size and weight of the mini-storage roots to 5-10 g (Mc David and Alamu, 1979; Martin, 1986).

These mini-storage roots can be stored for 10 to 12 months at temperatures of 20-24^{o}C and 75% of relative humidity.

Sweetpotato multiplication by sprouting storage roots in beds

Sweetpotato storage roots have adventitious buds that originate sprouts. These buds can be induced to produce sprouts. This characteristic is very useful in those areas where the weather has cold seasons or there are other sanitary reasons that do not allow a continuous multiplication of the collection using stem cuttings. Both yield and quality of the storage roots are not affected by the source from which the stem cuttings were obtained either storage roots or from the stems (El-Kattan & Stark, 1949).

The sprouting of the storage roots can be induced in recently harvested roots or in those that have been in storage for several months at 12-15°C and a relative humidity of 85-90%.

Pre-sprouting storage roots

The sweetpotato storage roots should be pre-sprouted to obtain a maximum number of stem cuttings for multiplication in the field. The pre-sprouting is the process by which the "seed" storage roots are conditioned to produce sprouts before they are placed in beds. Some refer to this as "waking up" the sweetpotato after they have been asleep in storage under controlled temperature (Wilson & Collins, 1987).

The storage roots to be used for sprouting in beds should be previously selected, discarding those that show rot symptoms. The storage roots should be of medium size, previously treated with some fungicide and bactericide, and placed in containers within the storage room with a temperature of about 25°C and approximately 90% of relative humidity, and with good ventilation. A good rule of thumb is that if a match will not stay lit there is probably not enough oxygen for the sweetpotatoes (Wilson & Collins, 1987). High relative humidity is obtained by watering the storage room floor twice a day.

The pre-sprouting is for approximately 15 days, during which are obtained 1-2 cm sprouts depending on the conservation of the storage roots. Under the environmental conditions of the south of United States, pre-sprouting is for approximately 3 weeks at 31-33°C and with 80-90% of relative humidity. This is a standard method to increase the production of plants for multiplication by stem cuttings (Deonier & Kushmann, 1960; Hall, 1993; Wilson & Collins, 1987).

Construction of beds

The beds for sprouting can be built with any type of material like bricks, stones or wood, depending on the area where the collection is maintained. It is recommended to use rectangular beds of approximately 1 m wide, a variable length according to the number of accessions in the collection, and about 15-20 cm deep. For best results, choose as a substrate only well drained and light soils that have had no sweetpotato on them for several years. Another substrate consists of a soil rich in organic matter that has been previously disinfected. Good results are also obtained with a substrate of sand obtained from a river, which is washed in running water and then disinfected with a water steam at 100°C for 4 hours. This washing-up of the sand allows eliminating any salts that can cause toxicity in the plants.

Planting the storage roots in beds

The storage roots are planted in beds preferably when the environmental temperature is relatively high (about 25°C) to induce fast sprouting. Covering the beds with a transparent plastic can

increase the temperature within the bed. However, this plastic should be removed when the sprouts begin to break the ground.

During the planting it is very important to handle the storage roots very carefully to avoid damage and cause wounds that could be the entrance for pathogens that cause rots. Similarly, the sweetpotato handling should be made in such a way to avoid breaking the sprouts. The storage roots are placed in the substrate leaving a space between them, deep enough to cover completely with a layer of substrate of about 5 cm above them. It is important that the storage roots of the same accession have more or less the same size so that all of them will be placed at the same depth. The planting of different sized storage roots of the same accession will result in an unequal coverage with the substrate (Steinbauer & Kushman, 1971).

Generally it is sufficient to plant 5 storage roots to obtain the necessary stem cuttings to plant an observation plot of more than 40 plants per accession. These five storage roots are planted in a single row per each accession in the collection and they are spaced at 15-20 cm between them to cover the width of the bed. In any case, at least 5 cm of separation between storage roots of the same accession should be kept. The separation between rows of the storage roots of different accessions should be about 25 cm.

After planting each accession, a plastic label should be placed at the top of each row with the identification number and the plot number where it will be planted in the field. Finally, the beds are watered very lightly to compact the soil over the storage roots. Before the breaking up of the sprouts in the surface of the bed very little water is needed. Afterwards, watering will be slight but very frequent to favor a fast plant development.

Cutting the stems for propagation in the field

Depending on the environmental temperature during sprouting in the beds, the emergency of the first sprouts will take place about a week after planting. Generally, the sprouting is faster when pre-sprouted storage roots are used. Approximately after three weeks from planting, most accessions will have plants. The number of stems produced by each storage root is variable and depends on the cultivar.

After approximately 45 days, depending on the environmental temperature and moisture of the substrate, plants will be big enough to get stem cuttings of about 25 cm for planting in the field. These plants will be re-sprouted and one can obtain more stem cuttings if they are needed for re-planting.

A single storage root can produce more than 20 stem cuttings for planting in the field. In commercial plantings in which fertilization is

applied to the beds, the production is from 600-800 stem cuttings per square meter (Bianchini & Boy, 1990).

Effect of the proximal dominance of storage roots

During the sprouting of the storage roots, in general, one can notice a higher production of the sprouts in the proximal part of the roots (i.e. the part which is close to the insertion of the root stock with the stem), this is known as proximal dominance. Depending on each cultivar, this physiological behavior will affect the sprouting speed of the buds located in the central and distal part of the storage root (Cooley & Kushman, 1938).

It has been demonstrated that breaking of the proximal dominance increases the production of the sprouts in the storage roots (Welch and Little, 1966). There are two methods to induce breaking this proximal dominance. One is a mechanic method that consists in cutting the storage roots in transverse sections to get 3-4 pieces. The other method is chemical and the products used are ethylene chlorohydrin, chloroethyl phosphoric acid, or dimethyl sulfoxide. The size of storage roots affect substantially the size of the sprouts, the larger storage roots will produce larger sprouts (Kays & Stutte, 1979).

Genetic variation in sweetpotato propagules

Although vegetative propagation is considered to preserve genetic integrity, it has been reported some variability of plants reproduced using different propagation methods. Thus, Huett (1982) reported storage root yield differences between plants obtained from storage root adventitious sprouts versus those from vine cuttings. Templeton-Somers and Collins (1986) also reported phenotypic variability between plants derived from vine cuttings from in vitro plants versus those from storage root adventitious sprouts. They also reported lower storage root skin and flesh color mutations in plants derived from stem cuttings. Using RAPD markers, Villardon and LaBonte (1996) reported that clonal plants derived from preexisting meristematic regions (for example, those from stem cuttings) are more genetically uniform than plants derived from storage root adventitious sprouts.

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Section 2.4

Tissue culture of *Ipomoea batatas*: Micropropagation and maintenance

Rolando Lizárraga, Ana Panta, Nelson Espinoza, John Dodds (Reproduced from CIP Research Guide 32)

Tissue culture allows the rapid clonal propagation of a large number of plantlets over a short period, as well as the maintenance of germplasm under controlled conditions in small spaces and with reduced labor requirements.

This document describes the advances, methodologies and materials used for tissue culture at the International Potato Center (CIP). It analyzes isolation, micropropagation, and long term storage techniques.

Sweetpotato (*Ipomoea batatas* (L.) Lam.) ranks seventh among the world's food crops according to FAO statistics (1978).

Advantages of tissue culture

CIP maintains a sweetpotato germplasm collection of over 5,000 accessions. Their clonal maintenance on the field is expensive and involves the risk of loss due to infectious diseases or unfavorable climatic conditions. Thus, in vitro maintenance presents the following advantages:

- lower labor costs,
- absence of field infections,
- protection against unfavorable climatic conditions,
- timely access to material under maintenance,
- timely access to material for pathogen clean-up,
- permanent availability of (when pathogen tested) material for propagation and exportation.

To date CIP maintains 2,800 in vitro accessions in its sweetpotato germplasm collection.

The *I. batatas* (L.) Lam, species is variously known in different parts of the world as batata, batata doce, boniato, camote, cumar, and sweetpotato. We shall hereafter refer to *I. batatas* as sweetpotato.

Introduction of in vivo material into in vitro

The in vivo mother plant must come from the greenhouse. It should be two to three months old, in excellent health conditions, and free of lateral buds that are too sprouted (at any rate, these must not be included.). The stems are excised from the mother plant and the leaves removed. Part of the petiole should be left to cover the buds. Stem cuttings 2 to 3 cm long each must include an axillar bud and a portion of the internode behind the bud for ease of manipulation. Before sending them to the in vitro laboratory, the stem cuttings are treated with a wide spectrum acaricide (that will destroy the acari at any stage of development) such as Morestan-Bayer (Chinometonat) at 0.5% for 10 minutes. The acaricide is next rinsed away by washing the stems in running water. They are then placed in a clean bottle covered with a petri dish until starting surface disinfection.

To start surface disinfection of the stem cuttings, remove the water from the container, add 96% alcohol and let stand for two seconds. Next, remove the alcohol and immediately add a 2.5% solution of calcium hypochlorite (brought to pH 8 by addition of HCl). Sodium hypochlorite or chlorine (bleach) may also be used. If possible, add a few drops of a dispersing-adhering agent such as Tween 20 or 80 (4 drops/l of solution). The bottle is then placed in the laminar flow transfer chamber. After 15 minutes under sterile conditions, the hypochlorite is eliminated, by washing three times with sterile water.

To reduce phenolization of the explants, after the last rinse they are left in a sterile 100 ppm solution of ascorbic acid before proceeding to excision.

Under these conditions, proceed to excise the buds by eliminating the largest possible number of leaflets and leaf primordia. The excised portions should be as small as possible. The optimum size is 0.6 mm but the explant may be bigger if no adequate excision instruments, such as a stereoscopic microscope, are available.

The buds are planted in an MMB-I culture medium and are kept there for 15 days. They are then transferred to an MMB-II medium where the plantlets will grow over a period of 30 to 60 days. Propagation by individual nodes may then be carried out in an MPB medium.

In the first propagation stage, 16 x 125 mm tubes are used. In the second stage, either 18 x 150 mm or 25 x 150 mm tubes may be utilized.

Due to the fact that in these cases meristematic portions larger than 0.6 mm are used, saprophytic bacteria mainly may contaminate some material. If so, proceed in either of two ways.

1. If a stereoscopic microscope is available, excise meristems between 0.4 and 0.6 mm long, plant them in the introduction medium (MMB-I) and transfer them weekly to the MMB-II medium.
2. Eradication of bacteria or yeasts may be attempted by adding antibiotics to the medium. For bacteria it is recommended to use Rifampicine (Rimactan 300 CIBA) at 400 ppm.

A concentrated (12,000 ppm), filter-sterilized solution of Rifampicine is used to soak small (5 x 5 mm) squares of filter paper which are allowed to dry in the laminar flow chamber. Approximately 0.03 cm^3 of concentrate solution is used for every square of filter paper. They are best if used before seven days for they progressively lose they effectiveness. This process must be carried out under aseptic conditions. The paper squares are introduced into the culture medium with the planted bud, which must be transferred to a fresh medium with another antibiotic paper every 3 to 5 days. Other antibiotics such as Cefotixine (Mefoxin Merck) in doses of 500 ppm may also be used.

For contamination by yeast it is advisable to use 0.25 to 0.5 ppm doses of Amphotericine B. The filter paper procedure described above will be used in all instances.

Meristem isolation and culture

The meristem is a tissue made of cells under division and is the active growth point of the bud. The dome of the bud contains the meristematic cells and is surrounded by foliar primordia and primary leaves. The meristematic cells divide and form new tissue. Nutrition of the dissecred section is provided by the artificial medium.

The isolation of the meristematic zone under aseptic conditions and its culture in an adequate nutritive medium allow plantlet development with a differentiation pattern similar to that of a normal plant.

The aseptic dissection of the meristem for virus eradication is a delicate process requiring skill. Photo 1 shows a photographic sequence of the dissection procedure. The steps involved are:

The plant stems are cut in segments including a node and the corresponding axillar bud. The material is disinfected with Morestan (Bayer) and sodium hypochlorite as for the introduction of in vitro material (see page 2).

After rinsing in distilled sterile water, place the material under the dissection microscope and use a needle or surgical knife (bistoury) to remove the leaves around the growth point until only the cupule and two or three foliar primordia are left.

The cupule and foliar primordia are dissected with the bistoury and transferred to culture medium MMB-I. The dissected meristem is transferred weekly to a fresh MMB-II medium. After 6 to 8 weeks the meristem will develop into a plantlet. The plantlets are now ready for subculture in the propagation medium (see section on Culture Media).

Thermotherapy

At CIP before excision of the meristems, the plants undergo a month thermotherapy at 38 C for 16 hours and at 32 C for eight hours under constant light conditions. This high temperature treatment has increased the efficiency of the production process of virus free material. After thermotherapy either axillar or apical meristems may be used indistinctly.

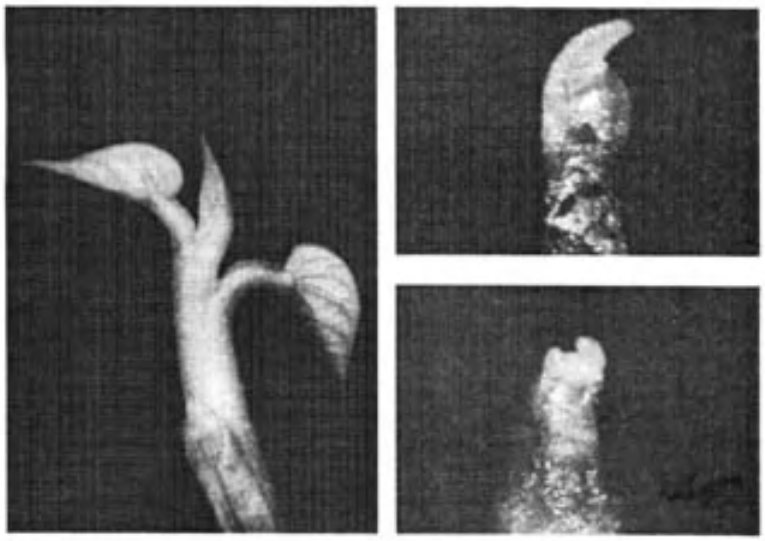

Figure 1. Photographic sequence of meristem dissection:
A: Isolated and disinfected apical bud
B: Dissection after removal of primary leaves
C: Meristem with two foliar primordia

Micropropagation

The purpose of micropropagation is to obtain a large number of clonal plants in a short time. The following methods are used at CIP:

Propagation by nodes

This is based on the principle that the node of an in vitro plantlet placed in an appropriate culture medium will induce the development of the axillar bud resulting in a new in vitro plantlet. It must be noted that this type of propagation is based on the development of a pre-existing morphological structure. The nutritional-hormonal condition of the medium plays a simple role in breaking the dormancy of the axillar bud and promoting its rapid development. The propagation medium described in the section on Culture Media is used.

Callus formation and plant regeneration must be carefully avoided because they tend to affect the genetic stability of the genotype.

The plantlets grow under long day conditions (16 hours of light at 45 m $E/m^2/seg^2$ or 3,000 lux) and at temperatures ranging from 25° C to 28° C. Under these conditions micropropagation is fast. Each node will develop into a plantlet occupying the full length of the test tube. The plantlets will be ready for subculture after six weeks (Photos 2 and 3).

Figure 2. Plantlet growing from a node

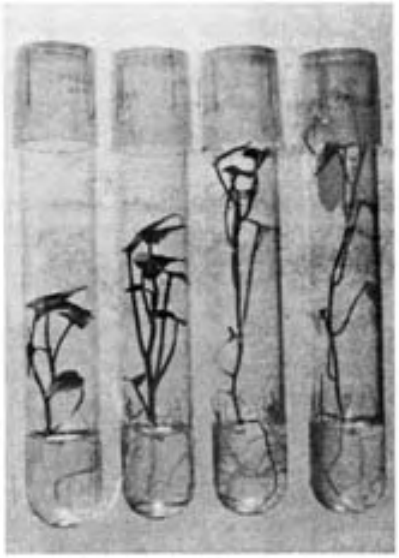

Figure 3. Sequence of in vitro development

The resulting sweetpotato in vitro plantlets may be easily transplanted to in vivo conditions either in small pots or directly to field beds (Photo 4).

Propagation by stem cuttings in liquid medium

As with the potato, it is possible to micropropagate sweetpotato in bottles containing a liquid medium (Photo 5). Stem cuttings with 5 to 8 nodes are prepared by removing both the apex and root of the plantlet to be propagated. The cuttings are placed in a liquid medium containing gibberellic acid to break the dormancy of the stem cutting's axillar buds. The nodes will sproute and new plantlets develop over a

period of 3 to 4 weeks. The plantlets may be used as initial material for simple node propagation or once again for propagation with stem cuttings in liquid medium, depending on program needs. Shaking of cultures may accelerate and promote the development of new plantlets. However, this is not essential.

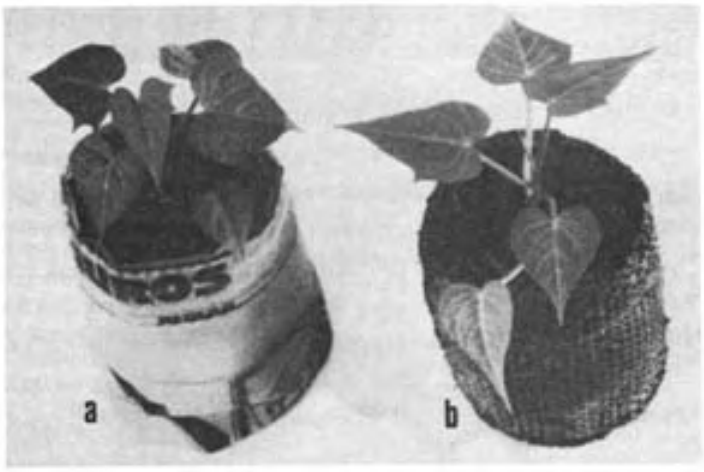

Figure 4. Plantlets after transfer to peat-moss pot
a: Homemade peat-moss pot (newsprint)
b: Commercial peat-moss pot (jiffy 7)

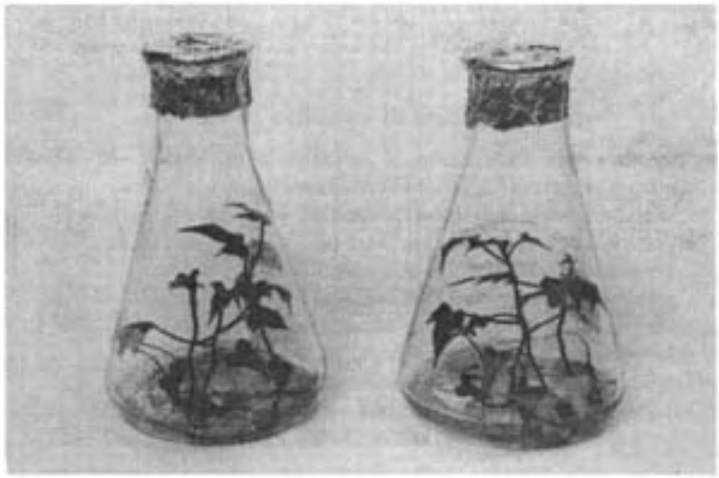

Figure 5. Liquid medium culture for rapid propagation

Long term maintenance

Long term maintenance is important for propagation and conservation itself. For clonally propagated cultures it is important that every propagule be free of even the slightest genetic alterations that may

build up from one generation to the next and result in major changes affecting uniformity and production.

For the maintenance of germplasm clones, it is crucial to conduct a detailed analysis of the culture's genetic stability. The clonal storage of germplasm consists on the maintenance of specific gene combinations (genotypes). If a plantlet's genetic combination changes during storage, the validity of the storage techniques must be examined. The capacity to detect genetic changes during propagation and maintenance depends on the methods used.

In many germplasm collections the stored genotypes are routinely evaluated for the morphological characteristics of the plantlets growing under controlled conditions. If the plants show new morphological characteristics (e.g. leaf shape, or storage root color) some genetic changes are obvious. However, genetic changes such as virus resistance may not be detected through the observation of morphological changes.

Biochemical methods are now used to study the genetic stability of both potato and sweetpotato. They are the analysis of soluble protein and isoenzyme patterns. Although these methods are highly effective in determining changes in genetic products, they do not allow directly to determine changes in the genes.

New methods such as Restricted Fragment Length Polimorphism (RFLP) are considered to be more sensitive ways of determining genetic changes. It is important that germplasm banks and seed programs use the most effective methods in determining the genetic fidelity of the propagation and maintenance systems.

Growth restriction media

After many years of research, propagation media for sweetpotato have been developed that optimize rapid in vitro growth. However, maintenance requires limiting growth to a minimum while maintaining culture viability. Use of growth restriction media allows to maximize the interval between transfers (subcultures) of in vitro plantlets. At CIP, transfer of most sweetpotato material under maintenance is carried out once a year, and in some cases once every year and a half.

Laboratory experiments aimed at limiting in vitro growth of sweetpotato include the use of hormonal growth retardants such as abscisic acid (ABA), growth inhibitors such as B995 or chloride chloride (CCC), as well as osmotic regulators with addition of low assimilation sugars such as manitol or sorbitol.

The difficulty involved in this type of study is that under these conditions genotypes react differently. Studies of germplasm collections in vitro maintenance should aim at developing maintenance media broad enough for a large variety of genotypes.

Also, the storage medium should not allow callus induction that may result in genetic alterations.

Many storage methods have been reported for sweetpotato. At present, the method described in the next section is used at CIP.

Restriction of storage temperature

The growth of in vitro plantlets may be restricted reducing incubation temperature. Adequate in vitro growth of sweetpotato can be obtained with temperatures between 28° C and 30° C. At 8° C, survival time is less than one month. For genotypes studied to date, 15° C seems to be the optimum temperature. However, this has to be confirmed yet.

As with other in vitro cultures such as cassava and potato, low temperature and growth retardants may be used simultaneously. For now, the combined use of osmotic stress and low temperature (15° C) appears as the best and least costly way of maintaining sweetpotato germplasm collections.

Culture media

Culture media used in the work reported here are based on Murashige-Skoog (1962) and Gamborg B-5 (1968) salts.

Medium for in vitro introduction (MMB-I)

- Calcium pantothenate 2 ppm
- Gibberellic acid 20 ppm
- Ascorbic acid 100 ppm
- Calcium nitrate 100 ppm
- Putrescine HCl 20 ppm
- L-Arginine 100 ppm
- Coconut milk 1%
- Sucrose 5%
- Agar or 0.7%
- Phytagel/Gelrite 0.25%

Medium for transfer of meristems or buds (MMB-II)

- Calcium pantothenate 2ppm
- Gibberelic acid 15 ppm
- Ascorbic acid 100 ppm
- Calcium nitrate 100 ppm

- Putrescine HCl 20 ppm
- L-Arginine 100 ppm
- Saccharose 5%
- Agar or 0.7%
- Phytagel/Gelrite 0.25%

Propagation medium (MPB)

- Calcium pantothenate 2 ppm
- Gibberelic acid 10 ppm
- L-Arginine 100 ppm
- Ascorbic acid 200 ppm
- Putrescine HCl 20 ppm
- Sucrose 3%
- Agar or 0.8%
- Phytagel/Gelrite 0.3%

Maintenance medium (MCB)

- Glucose 2%
- Sorbitol 2%
- Putrescine HCl 20 ppm
- Phytagel/Gelrite 0.4%

A pH 5.8 is used in all media.

Note: These culture media were prepared to attain maximum uniformity in a collection including a large number of varieties.

When few varieties are involved, it is advisable to use simple culture media such as Murashige and Skoog salts with the addition of gibberellic acid and sucrose.

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ANNEX : Culture Media for in Vitro Conservation of Sweetpotatoes used by CIP in 1997. Judith Toledo

ANNEX
CULTURE MEDIA FOR IN VITRO CONSERVATION OF SWEETPOTATOES USED BY CIP IN 1997

Judith Toledo

MPB-BASE

Distilled water	200 ml
Ascorbic acid	8 g
Arginine	4 g
Putrescine	0.8 g
Giberellic acid	0.4 g
Calcium Pantothenate	0.08 g

pH 5.8

Dispense in tubes of 20 ml.
Use 5 ml/l

BASE STORE

Distilled water	500 ml
Glycine	0.30 g
Nicotinic acid	0.75 g
Pyridoxine	0.75 g
HCL Thiamine	0.60 g

pH 5.6

Dispense in tubes of 20 ml.
Use 5 ml/l

Propagation Media
(1 Liter)

MS	1 bag
Base MPB	5 ml
Sucrose	30 g
Phytagel	3.5 g

pH 5.8

Conservation Media 21.5
(Dextrose 2%, Sorbitol 1.5%)
(1 liter)

MS	1 bag
Base Store	5 ml
Espermidine	2 ml
Sorbitol	15 g
Dextrose	20 g
Phytagel	3.5 g

pH 5.8

Conservation Media 22
(Dextrose 2%, Sorbitol 2%)
(1 liter)

MS	1 bag
Base Store	5 ml
Putrescine	2 ml
Sorbitol	20 g
Dextrose	20 g
Phytagel	4 g

pH 5.8

Conservation Media 522
(Sucrose 0.5%, Sorbito 2%, Manitol 2%)
(1 liter)

MS	1 bag
Base Store	5 ml
Espermidine	2 ml
Manitol	20 g
Sorbitol	20 g
Sucrose	5 g
Phytagel	3.5 g

pH 5.6

Conservation Media 20.5
(Sucrose 2%, Sorbitol 0.5%)
(1 liter)

MS	1 bag
Store	5 ml
Espermidine	2 ml
Sorbitol	5 g
Sucrose	30 g
Phytagel	3.5 g

Section 2.5

Transport, receipt, and propagation of in vitro sweetpotato plantlets

John Dodds, Ana Panta, James Bryan

(Reproduced from CIP Research Guide 38)

Tissue culture materials consist of small, aseptic plantlets growing on a synthetic nutrient medium. The aseptic nature of this material makes it an ideal method for international exchange of germplasm as it minimizes the risk of transmitting fungal and bacterial diseases.

This document contains information for the recipient of in vitro plantlets on the handling procedures to be followed for further micropropagation or transfer to non-sterile growing conditions.

Transport of tissue culture material

1. Packing. The material is packed in polystyrene in a cardboard container. Each package contains several small glass test tubes, each with three well-developed plantlets. Extra agar media is added to prevent damage from movement during shipment (Photo 1).

Figure 1

The test tubes are capped with plastic covers and sealed with parafilm to prevent entry of contaminants into the cultures and loss of water from the medium.

2. Shipment. Whenever possible, tissue cultures are hand-carried to ensure rapid transport. When this is not feasible, the fastest possible method is usually airfreight. In vitro plantlets can survive two to three weeks without light.

3. Handling after arrival. The cultures should be cleared from customs as quickly as possible. When advance notice of the shipment is known, alert the customs officials of its expected arrival. Carefully remove the test tubes from the package in a laboratory or clean room. Do not open the tubes. Do not remove the plantlets.If the plantlets have become yellow, place the test tubes under diffused light for about 1 week in a clean room.

In vitro shoot cultures are free from diseases. Work under clean conditions according to the following description (steps 4 and 5 of he following section on "Transfer to Planting Materials") to prevent contamination during and after unpacking.

4. Use of tissue culture material. The plantlets can be used in two different ways:

- Transfer to planting mix
- Micropropagated

Transfer to planting mix

1. Materials
    - Peat moss
    - Fine sand (1 mm diameter)
    - Aluminum foil
    - Clay pots (8 to 10 cm diameter) or jiffy pots
    - Larger pots (20 cm diameter)
    - Distilled water
    - 1% calcium-hypochlorite solution
    - 70% alcohol solution
    - Strong soap
    - Fertilizer with high content of phosphorus (5-50-17) or (12-12-12)

2. Mix peat moss and sand (1:2 volume).
    - If an autoclave is available, fill the pots with peat moss/sand mix, cover them with aluminum foil, and sterilize for 1 hour. If an autoclave is not available wash the clay pots (jiffy pots are already sterile) with detergent, rinse them well with running water, and sterilize the planting mix and some additional sand separately by any other means (heat, steam, fungicides, etc.).
    - Take the pots and the in vitro culture to a clean bench that is protected from air currents, dust, dirt, insects, or other contaminants.
    - Wash your hands with strong soap and 1% calcium-hypochlorite solution. Then rinse hands in 70% alcohol.
    - Irrigate the pots with a small amount of water.
    - Prepare the pot that is to receive the plantlet by making a hole in the center of the peat moss/sand mix with a clean stick or pencil.
    - Before removing the plantlets, disinfect the outside of the test tube using a piece of cotton or cloth moistened with 70% alcohol to reduce the risk of contamination.

- Using clean fingers, remove the parafilm and the plastic cover from the test tube. Work with one tube at a time.

3. Gently pull the plantlets with the agar out of the test tube using sterilized forceps (flamed to red heat and cooled) (Photo 2).

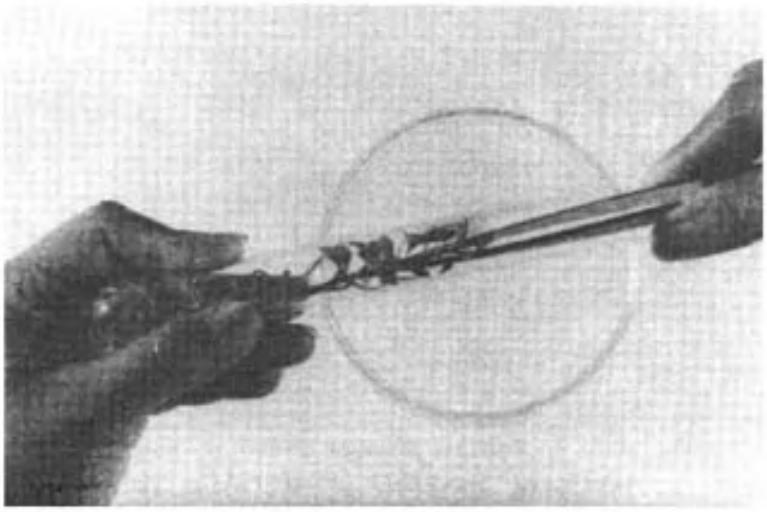

Figure 2

4. Wash the agar from the roots by gently immersing them several times in sterilized water, trying not to wet the rest of the plantlet.
5. Plant each plant individually in the holes in the potting mix with the roots plus one or two nodes below the surface (Photo 3).
6. Place the sterilized sand around the plantlet and press lightly to keep the plantlet straight in the pot.
7. Place the plant into a humid chamber during 48 h. Remove the humid chamber and wait until the roots are established (about 10 days).
8. Keep the pots in a clean location, at 25 to 27° C with 14-16 hours illumination.
9. Until the plants are well rooted irrigate lightly with tap water if it has a low salt content; otherwise use demineralized or rain water. Do not overwater.

Figure 3

10. When roots are established, you may dissolve supplementary nutrient in the irrigation water. Commercial peat moss often contains fertilizer, thus less additional nutrient may be required.
11. Gradually expose the plants to the normal atmosphere by removing the beakers for short periods each day.
12. Once the plants are established, transfer to larger (e.g. 20 cm diameter) pots. Be careful not to break the roots. When the plants are well rooted, normal fertilizer can be dissolved in the irrigation water. At CIP we use 5 g N:P:K at 12-12-12 per liter water. Apply 50-100 cm^3 per 20 cm diameter pot. Again, do not overwater

Micropropagation

1. Materials and equipment
    - Culture medium (see next two following sections)
    - Test tubes
    - Plastic caps or cotton wool
    - Forceps
    - Scalpel

- Parafilm
- Alcohol lamp
- Alcohol 70%
- Autoclave
- Sterile work area ("microvoid")

2. Prepare the nutritive growth medium according to the procedure given in the section on Medium to Sweetpotato Micropropagation.
3. Dispense 4 cm^3 of the medium in each test tube. Cap the tubes with plastic caps or cotton wool plugs and autoclave them for 15 minutes. Keep the test tubes vertical while the agar sets.
4. Working under sterile conditions (sterile area or "microvoid") follow steps 8 to 10 of previous section.
5. Transfer the plantlets from the test tuber to a sterile petri dish and make nodal cuttings using sterile scalpel and forceps. Each nodal cutting consists of a 0.2-0.5 cm stem segment with an axillary bud (Photo 4).

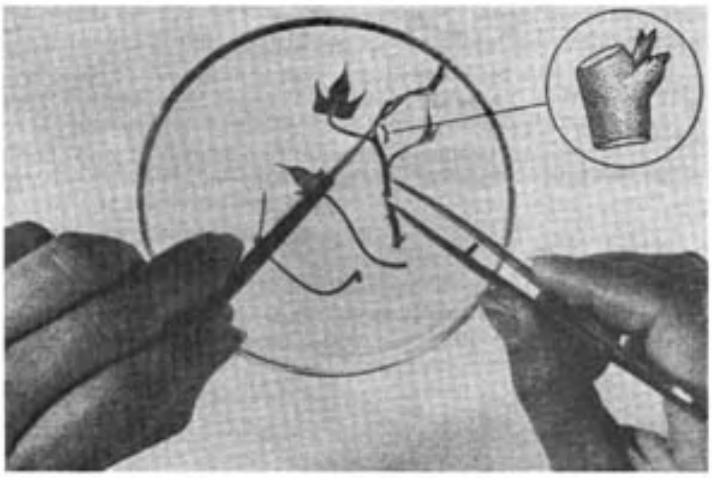

Figure 4

6. Place each nodal cutting in a test tube. Ensure that each cutting lies on the agar surface with its axillary bud pointing upwards (Photo 5). Place 1 or 2 cuttings per test tube.

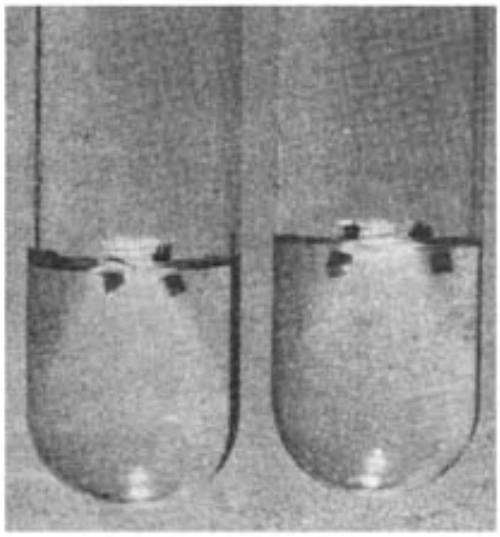

Figure 5

7. Close the test tubes, seal with parafilm, label and place in a clean area where the room temperature is 25-27° C. Give 45m $E/m^2/seg^2$ or 3,000 lux illumination for 14-16 hours each day.
8. The axillary bud of each nodal cutting grows into a new plantlet within 2-4 weeks and is ready for transplanting to pots as previously described, or for further micropropagation (Photo 6).

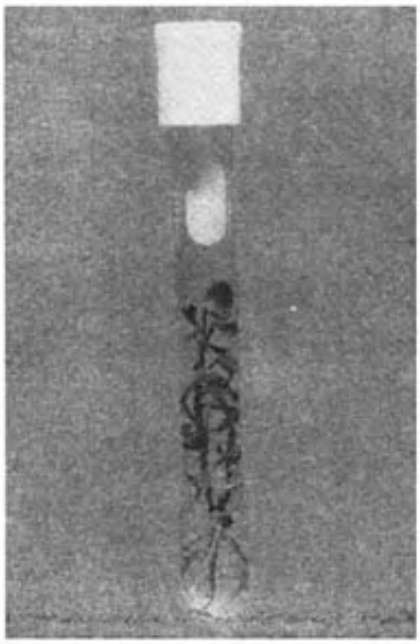

Figure 6

Murashige-Skoog medium (modified)

Prepare the stock solutions in four separate parts:

- Salts
- $MgSO_4$
- Iron
- Vitamin

Salts stock solution: Dissolve each in 200 cm3 glass distilled water.

- NH_4NO_3 : 35.0 g
- KNO_3 : 40.0 g
- $CaCl_2$ $2H_2O$: 9.0 g
- KH_2PO_4 : 3.5 g
- H_3BO_3 : 0.1 g
- $MnSO_4$ $4H_2O$: 0.5 g
  ($MnSO_4$ H_2O : 0.4 g)
- $ZnSO_4$ $7H_2O$: 0.2 g
  ($ZnSO_4$ H_2O : 0.01 g)
- KI : 0.02 g
- Na_2MoO_4 $2H_2O$: 0.005 g

Dissolve 5 mg (0.005 g) of the following salts together in 10 cm^3 of water; add 1 cm^3 of this solution to 200 cm^3 water for the stock solution.

Mix the ten individual salt solutions together to make 2,000 cm^3 of the salt stock solution.

$CuSO_4$ $5H_20$ and $CoCl_2$ $6H_2O$

Mix the ten individual salt solutions together to make 2,000 cm^3 of the salt stock solution.

MgSO4 stock solution:

$MgSO_4$ $7H_2O$ 3.7 g in 100 cm^3 distilled water

Iron stock solution:

Na_2EDTA : 0.75 g

$FeSO_4$ $7H_2O$: 0.55 g

Dissolve $FeSO_4$ $7H_2O$ in 20 cm^3 distilled water; Na_2EDTA in 20 cm^3 distilled water heating it up. Mix the solutions, cool, and make up to 100 cm^3 with distilled water.

Vitamin stock solution:

Thiamine HCl 40 mg in 100 cm^3 distilled water

Medium preparation:

Prepare 1 liter of the Murashige-Skoog basic medium by mixing the stock solutions with additional materials in the following proportions:

- Salts 100 cm^3
- $MgSO_4$: 10 cm^3
- Iron 5 cm^3
- Vitamin 1 cm^3
- Inositol 100 mg
- Gibberellic acid 0.25 ppm
- Calcium pantothenic acid 2.0 ppm
- Sucrose 3.0%
- Agar 0.8%

Autoclave for 15 minutes.

Medium to sweetpotato micropropagation

The medium used for this work is based on the salts Murashige-Skoog (1962). Prepare 1 liter of Murashige-Skoog medium with additional nutrients in the following proportions:

- Calcium pantothenate 2 ppm
- Gibberellic acid 20 ppm
- Ascorbic acid 100 ppm
- Calcium nitrate 100 ppm
- Putrescine HCl 20 ppm
- L-Arginine 100 ppm
- Coconut milk 1%
- Sucrose 5%
- Agar or 0.7%
- Phytagel/Gelrite 0.25%

Autoclave for 15 minutes.

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Section 2.6

Methods to induce flowering in sweetpotato

Daniel Reynoso, Zósimo Huamán, César Aguilar

Introduction

Within a sweetpotato collection, a wide variation is generally observed in the flowering habits of different accessions. Under normal sowing conditions, some cultivars do not flower, or have scanty flowering and others flower profusely.

Flowering is indispensable in order to obtain sexual seed from the accessions maintained in a collection, both for long term gene conservation or for use in breeding. Many flower characteristics can help us to verify if two or more accessions are duplicates or not. One of these characteristics, for example, is the degree of stigma exertion over the stamens, which should be equal in all accessions of the same duplicate group. Another characteristic is cross compatibility,

because all accessions of the same duplicate group must have the same cross compatibility.

The production of sexual seed in sweetpotatoes is limited by genetic and environmental factors. The most important genetic factors are: duration and intensity of the reproductive period, spore incompatibility, partial or total fertility or sterility, and the structure of the ovary (2 carpels and 2 locules). The hexaploid nature of this species (2n=6x=90) significantly influences the last three factors, while its vegetative propagation favours the preservation of mutations. The most important environmental factors are: the duration and light intensity, temperature, water supply, nutritional balance, and pest and disease attacks.

Several techniques have been developed to promote not only sweetpotato flowering but also fruit and seed production. These are short photoperiod, moderate temperature, limited water supply, grafting, trellises, growth regulators, overwintering, vine girdling, pesticide sprays, soil fertilization, the “bouquet” method, and genetic selection. In practice, a combination of these methods is used, and commonly complemented with the determination of sexual fertlity and compatibility of the accessions to be included in seed production.

Short-day treatment

Flowering is induced or magnified by using photoperiods of 8-11.5 hours of intense light (Campbell *et al*, 1963; Eguchi & Gonzalez, 1989; Lam *et al*, 1959). Fruit set is higher with photoperiods of 11.5-12.5 hours (Miller, 1937). Flowering and fruit set are highest with temperatures of 20-25^{o}C and a relative humidity over 75% (Du Plooy, 1983; Srinivasan, 1977). Because of these conditions, pollinations are more successful in the early morning.

A short-day treatment can be accomplished by covering the plants with black plastic or other dark material from 5 pm-8 am, for example. The plants can be grown in pots within beds, inside or outside a screenhouse, or directly in the ground, except those that are grafted onto *Ipomoea nil* cv. “Kidachi Asagao” (Photo 1). Stem tips should be removed before starting the treatment to stimulate the development of axillary buds. Flower buds will appear about one month after the treatment is initiated. Once induced, plants can be moved to a screenhouse for hand-made pollinations or controlled interpollinations (Ellis *et al*, 1981), or to an isolated field. In some cases, this treatment must be repeated to assure continuous flowering.

Photo 1. Sweetpotato plants flowering using tripods as trellises

Grafting onto rootstocks with profuse flowering

Sweetpotato scions grafted onto rootstocks with profuse flowering, blossom early, and in a synchronized and profuse fashion (Photo 2). The most efficient rootstocks are *Ipomoea carnea* subsp. *fistulosa* Jacq. (Mart. ex Choisy), *Ipomoea nil* cv. “Kidachi Asagao”, var. *integriuscula* or var. *limbata* in warm climates, and *Ipomoea purpurea* var*. alba* in cold climates (Eguchi & Gonzalez, 1989; Folquer, 1978; Kobayashi & Nakanishi, 1982; Lam *et al*, 1959; Lardizabal & Thompson, 1988). Some sweetpotato clones may also be used as rootstocks, but with variable results (Campbell *et al*, 1963; Hsia & Ching-Kuan, 1956). In all cases, a 'cleft' type of graft is used with a 2-5 cm sweetpotato scion. The graft must be fixed with a small pin, and the plant covered with a plastic bag under shade for a week. After approximately 3 weeks, a short-day treatment could be initiated after pruning the tip of the scion. Best results are obtained when rootstock plants are young and the scions come from mature sweetpotato plants. In addition, both rootstock and sweetpotato mother plants should be treated previously with a short photoperiod. Finally, flowers of rootstock plants must be removed as soon as they appear.

Photo 2. Sweetpotato plants grafted onto *Ipomoea nil* cv. "Kidach Asagao" in middle flowering

Trellises, growth regulators and pesticide sprays

The use of trellises has a relative efficiency in flowering induction. Its effect is associated with the upward vegetative growth of the plants, higher exposure to light, lower attack of soil-borne pathogens and insects, and the facility to collect capsules. Structures such as tripods, stakes, wire meshes, etc., can serve as trellises (Miller, 1939; van Rheenen, 1965). Sweetpotato vines must be fixed to the trellises with strings or pins as they grow, and vegetative branches at the bottom eliminated in non-grafted plants.

Growth regulators reported to be efective for flowering induction in sweetpotatoes are gibberellic acid (GA_3 or GA_7), and etephon, (2-chloroethyl phosphonic acid), specially when combined with a short-day treatment and using scions from mature mother plants (Lardizabal & Thompson, 1988; Suge, 1977). A unique early spray (200 to 1,000 ppm) applied to the apex, or divided into fractions, is recommended.

In some environments, fungi such as *Fusarium moniliforme*, among others, and insects such as *Prodiplosis* sp., *Melanogromiza caerulea*, *Nezara viridula*, etc., attack immature sweetpotato anthers or seeds, in plants growing in the field. Their control with systemic pesticides increases flowering and seed set (Jones *et al*, 1977). However, these insecticides curtail the activity of insect pollinators.

Overwintering and vine girdling

Overwintering is mostly recommended for temperate zones. It consists in removing stem cuttings of 1 m long at the end of the summer, rooting them in water, planting them in 10-inch-pots (that also have a stake to support the vines), and transferring them to the greenhouse as soon as a danger of frost appears (Miller, 1939). Plants remain there until spring, when they are moved back to the field and planted with larger trellises. They will flower earlier and remain in the reproductive state throughout the growing season.

Vine girdling usually complements overwintering. It consists in making a slanting incision across the stem at about 15 cm from the ground, cutting vines about two-thirds through (Campbell *et al,* 1963; Miller, 1939; Hsia & Ching-Kuan, 1956). Re-girdling must be continued every 10 -15 days when weather conditions favor vegetative growth. Girdled plants flower earlier and produce more flowers than non-girdled plants. First flowers appear 30 to 45 days after girdling.

Mineral nutrition and the “bouquet” method

Conventional nutrient solutions do not stimulate flowering. Their enrichment with boron, magnesium and iron has a slightly positive effect, depending on the clone (Lardizabal & Thompson, 1988). Nevertheless, seed quality is negatively affected by any nutrient deficiency. An application of medium to low doses of nitrogen and potassium is recommended, and a high dose of phosphorus (van Rheenen, 1965). Under this nutrient regime, plants will be smaller, their vegetative period shortened, and their flowering habit stimulated, especially if the water supply is limited and other techniques to induce flowering are applied.

The 'bouquet' method is actually for seed production only, because it uses natural flowering, providing a suitable environment for pollination and seed maturity when external conditions are unfavorable. It consists in placing 3 cuttings, 80 cm long, containing a large number of flower buds, the first of which is about to open, in bottles filled with a water solution containing 5 cc/L of a 4-12-4 NPK stock solution 10% strong, or 3.5 cc/L of sulfuric acid (Miller, 1939). In any case, an extra supply of 2 ppm of boric acid is recommended (Otazu & Amoros, 1991). Solutions must be replaced weekly. Bottles are kept in a greenhouse to carry out pollinations. The amount of seed produced with this method is 2 to 3 times higher than with tap water only.

Genetic selection

Genetic selection for a profuse flowering habit is the most radical method to assure blossom in a set of bred parents. Clones are selected according to their flowering habit and seed set exclusively.

This should be done before a mass or recurrent selection scheme starts. The first set of parents should be induced to flower using some techniques outlined before to secure a balanced gene recombination. They may be divided into groups of 10 parents and then mated, following a diallel design, or simply left to open pollination. Seeds are mixed proportionally, e.g. 15 seeds per mating. Seedlings are then grown in an isolated field, using trellises, and left to open pollination. Only 10% of the plants (100 to 500) is kept for the next generation, and they are selected according to their seed productivity only. Seed is mixed in equal amounts or just bulked. A new lot for intermating is planted, and selection continues for 3 or 4 more generations, while remnant seed may be used for conventional tests or serve as stock. Finally, flowering and seed set will be higher, and gene recombination maximized. The gene frequency of other traits may have changed because of a non-deliberate selection, sample bias, or genetic linkage (Jones, 1966, 1972; Martin & Jones, 1971).

The success of populations generated in this way will largely depend on the original genetic variability and the efficiency of the selection scheme followed.

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Section 2.7

Methods to determine the fertility and compatibility of sweetpotato

Daniel Reynoso, Zósimo Huamán, César Aguilar

Introduction

Sexual reproduction allows the combination of desirable traits, such as high productivity, resistance to pests, diseases, and environmental stresses, and industrial or culinary quality, that are present in sweetpotato clones. Crossability among sweetpotato clones ranges from 0 to 100%. Because of incompatibility and limited fertility, lower values are more frequent and, therefore, constitute a serious barrier for genetic improvement and the conservation of the genetic resources of this crop.

Crosses between compatible parents seldom produce 4 seeds per capsule, which would be expected from an ovary with two carpels and two locules. Instead, they typically produce 1 or 2 seeds per capsule.

Determination of Fertility

Sweetpotato fertility is frequently from 20-50%, although total sterility is not rare (Burnham, 1967; Jos & Bai, 1978; Wang & Burnham, 1968). This limited fertility of gametes is due to abnormalities in meiosis associated with the hexaploid nature of this species (2n=6x-90). In fact, two of the three genomes show a high homology and the size of chromosomes is quite small (Oración *et al.*, 1990; Shiotani & Kawase, 1989; Ting & Kehr, 1953).

Male fertility can be easily estimated by staining pollen grains with an aceto-carmin glycerol solution (2%). From each parent, a minimum of 4 flowers is sampled and their anthers removed and brushed on a drop of the solution placed on a slide. Immediately after, a cover-slide is placed on top, avoiding the formation of air bubbles. Two samples from the same parent can fit on a single slide. Four hours later, stained and unstained pollen grains are counted using a common microscope of 40X (Photo 1). The percentage of male fertility is estimated by multiplying by 100 the quotient between the number of stained pollen grains divided by the total number of grains counted.

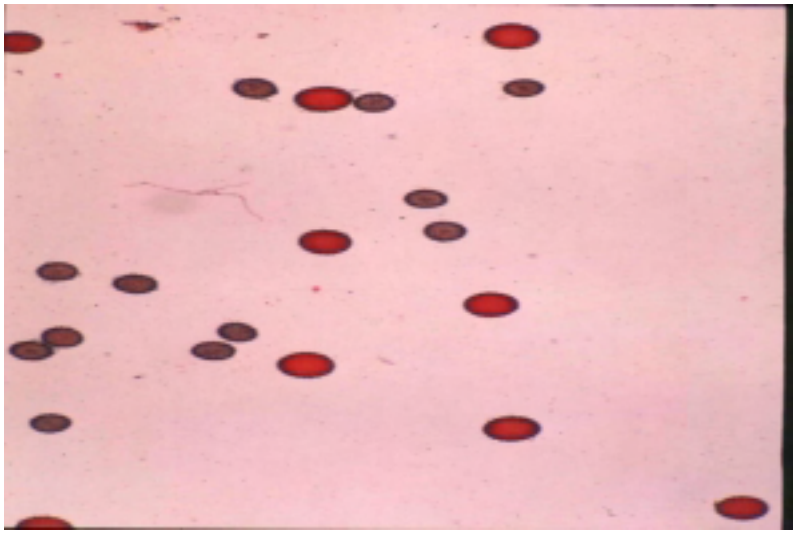


Photo 1. Stained and unstained sweetpotato pollen grains (40X).

Under laboratory conditions, pollen samples fixed in this way can last several weeks if kept horizontally within a cage.

Preparation of 120 ml of aceto-carmin glycerol (2%)

1. Pour 45 ml of acetic glacial acid into a beaker.

2. Increase to 100 ml with distilled water. Heat under a laminar hood.

3. Add 2 ml of aceto-carmin and stir. Continue heating until the solution boils.

4. Cool and filter twice with Whatman paper N° 4.

5. Add an equal volume of glycerol (~ 60 ml). Stir.

6. Pour the solution into an amber bottle with a dropper at the top.

7. Remove from the laminar hood.

8. Store in a refrigerator (5 °C).

Female fertility can be estimated by examining the number of ovules contained in ovaries. Ten flower buds per clone are collected on the day of anthesis, or the afternoon before. Sepals and corolla are removed. The pistil may be detached from the basal yellow glands or the ovary cut transversely. Then, ovules are squeezed out of the ovary and counted. Female fertility is estimated by multiplying by 100 the quotient from the mean number of ovules per ovary divided by 4 (Burnham, 1967).

Determination of incompatibility

Incompatibility in sweetpotatoes is sporophytic, and it is the main barrier to seed production (Martin, 1965; Martin & Cabanillas, 1966; Togari, 1942a, 1942b, 1942c). This means that most clones are self-incompatible, some matings are compatible and others not, and compatibility may be unilateral, that is, seed set takes place in one direction only (Vimala, 1989). Pollen grains with 3-nucleus, short-life span, and no germination over incompatible stigma or in vitro, as well as the dryness of the stigma, are features associated with sporophytic incompatibility (Brewbaker, 1957). On the other hand, the degree of stigma exertion over the stamens is not related to it (Venkateswarlu, 1980).

The Japanese system of classification of sweetpotato incompatibility groups, 18 at the present, is very practical (Nakanishi & Kobayashi, 1979). Clones within each group are incompatible whereas those from different groups are compatible. Representative clones from each group have been identified and are known as 'incompatibility testers'. They are used in crosses whose compatibility needs to be determined. Sweetpotato cultivars from Peru, Colombia and Brazil exhibit the highest number of incompatibility groups (10, 8 and 7,

respectively), followed by those from Mexico, the United States, the Philippines, the South-East Pacific Islands, and the remainder of Asia. Thus, sweetpotatoes from China and Japan show only 3 groups (A, B and C), and those from Thailand only 2 (A and B).

Incompatibility is determined by observing the development of the pollen tube using a fluorescence microscope (Martin & Ortiz, 1966). Flowers are emasculated the afternoon before anthesis and covered with a small hood made of glassine paper (Photo 2). Pollination is carried out on the day of anthesis by rubbing the anthers over the stigma. After pollination, the flower is covered again with the small hood, and labeled with the name of the parents and the date of crossing. Anthers from the same male can be placed in a Petri dish to facilitate their use and avoid contamination. Four hours after pollination, pistils are carefully removed. Thereafter, either of two staining techniques can be followed:

1. Staining with aniline-blue

Pistils from the same parent are dipped for one minute in small tubes containing 3 ml of a 4N NaOH solution. Later they are dipped in distilled water, dried slightly on a paper towel, and the stigmas are alternated in opposite directions on the same slide over a drop of aniline-blue (0.5%). They are then lightly pressed with a cover slide of 44 x 22 mm, avoiding air bubbles. Samples are identified, dated, and stored horizontally in a refrigerator for 12 to 18 h. Finally, pollen behavior is observed with a 40-to-100X fluorescent microscope (Photos 3 and 4). The disadvantage of this technique is that it is slow and requires gloves and other protective measures, because direct exposure to this stain can be dangerous, and samples must be observed immediately.

Photo 2. Emasculation of a sweetpotato flower bud.

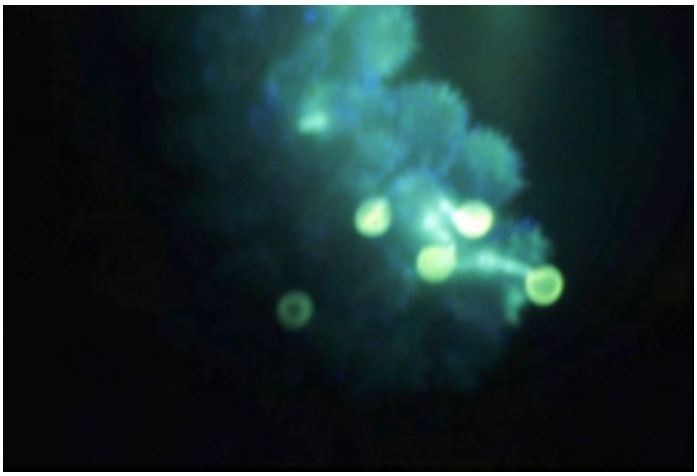
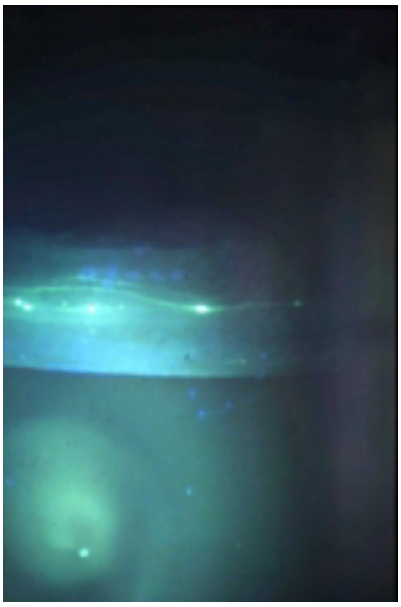


Photo 3. Germination of sweetpotato pollen grains over the stigma in a compatible mating (40X).

Photo 4. Pollen tube growth in a compatible mating (40X).



Preparation of 100 ml of a 4N NaOH solution

1. Weigh 16 g of NaOH.
2. Dissolve it in distilled water until completing 100 ml, within a laminar hood.
3. Pour in an amber bottle.
4. Remove from the laminar hood.
5. Store in a refrigerator (5 °C).

Preparation of 100 ml of aniline-blue (0.5%)

1. Dissolve 10 ml of glycerine in 90 ml of distilled water in a glass flask.
2. Add 0.71 g of K_3PO_4 within a laminar hood.
3. Add 0.5 g of aniline-blue. Cover the flask with aluminum foil. Stir.
4. Pour into an amber bottle.
5. Remove from the laminar hood.
6. Store in a refrigerator (5 °C).

2. Staining with Schreiter-Tiemann solution

Pistils from the same cultivar are dipped for 1 minute in a small tube containing 3 ml of Schreiter-Tiemann solution. Immediately afterwards, or several days later under cold storage, tubes are placed in a pan or flask half-filled with water which is boiled for 4 to 5 minutes, until pistils become glassy and flaccid. The contents of the tube are poured into a concave recipient. Pistils are carefully removed with a fine paintbrush and placed opposite each other on a slide over a drop of glycerine (50%). They are then squashed with a glass cover of 44 x 22 mm, avoiding air bubbles. Samples are identified, dated, and stored horizontally in the dark in a refrigerator for 12 to 18 h. Samples can last for several weeks or years if fixed with pure glycerine. As in the previous technique, pollen behavior is observed with a 40-100X fluorescent microscope. Another advantage of this technique besides the longer duration of samples, is that the solution can be reused 10 times, although it must be filtered when turbid.

Preparation of 1.2 L of Schreiter-Tiemann solution

1. Dissolve 2 g of aniline-blue in 100 ml of distilled water. Boil and filter.

2. Dissolve 20 ml of Tween 20 in 189 ml of distilled water.

3. Dissolve 49.53 g of $K_3PO_4.3H_2O$ in 700 ml of distilled water.

4. Dissolve 8 g of NaOH in 200 ml of water (1N).

5. Mix all four of above solutions in an amber bottle.

6. Store in a refrigerator (5 °C).

Pollen grains do not germinate over the stigma in incompatible crosses. In addition, other crossability barriers exist between the stigma and style, and between the style penetration and seed development (Martin & Cabanillas, 1966). Finally, the physiological status of the pollen is likely to play an important role in the reproductive process. Therefore, 'pollination tests' give more reliable information about the fertility and compatibility of sweet potato than other tests outlined so far.

It is recommended that from 10 to 25 selfings and biparental crosses they be made the season before massive seed production. Flowers will commonly drop 3 to 4 days after pollination in incompatible crosses. If the crosses are compatible, seeds will mature 25 to 55 days after pollination. A good indicator of maturity is when the pedicels dry out. Whatever technique is used, cross incompatibility is a useful feature to verify duplicate accessions of the same genotype.

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Section 2.8

Sweetpotato sexual seed management

Zósimo Huamán and Humberto Asmat

One of the objectives of a genebank is to maintain the viability of the sexual seed stocks of all the accessions in the collection for a long time. The appropriate management of the sexual seed of the sweetpotato and related wild species includes the elimination of impurities, their disinfection, the seed number determination, their drying for storage, their packaging and storing, and their germinability tests.

Elimination of impurities

The seed stocks received by the genebank usually contain some impurities that must be eliminated. However, besides the remains of the capsules, pieces of inflorescences and other kinds of strange particles, there are also seed mixtures of other species, seed damaged by insects, and empty, deformed, or mummified seed. The

cleaning of small seed lots is usually done by hand. However, for larger seed lots, the use of seed blowers helps to discard these impurities and also the empty seeds.

Separation of viable and non-viable seed by the flotation method

The separation of viable (non-floating) seeds from those that may be immature or non-viable (floating), is easily made by pouring the seed into a glass or plastic container of 200 ml capacity containing a solution of water and any commercial detergent. The solution is then stirred in a circular way producing a whirlpool to submerge all seeds. After some minutes, all floating seeds are discarded by pouring off part of the solution. The seeds that stay at the bottom are then poured off into a plastic sieve and placed on a paper towel to dry.

In spite of the light weight of non-floating seeds, they can germinate in vitro. However, it is recommended that, for the maintenance of plant genetic resources, only the well-shaped and matured seed should be stored.

Seed disinfection

Before seed disinfection, all seeds damaged by insects should be eliminated. They are easily identified by the holes produced at the time the insect exits from the seed.

All seed apparently healthy is treated with an insecticide that has fumigant properties, like Vapona (DDVP) used in cereal seed stores. Vapona tablets, of 30 g approximately, gasify when put in the environment. For disinfecting sweetpotato seeds, these are placed in a container that can be hermetically sealed (petri dishes, for example) and Vapona is cut in pieces of 1 cm^2 approximately, using 1 piece per 100 seeds. Finally, the containers are sealed and stored for at least 30 days. After treatment with Vapona, the seeds are examined in order to throw away those seeds with insect emergence.

VAPONA

Common Name: DDPV (ANSI), Dichlorvos (ISO, BSI).

Chemical composition. 2,2-Dichlorovinyl dimethyl phosphate, or 2,2-Dichloroethenyl dimethyl phosphate.

Chemical properties. As a tablet, it gasifies in the environment. Steam pressure is 2.9 x 10^3 mbar at 20°C. Boiling point is 117°C. It works by inhalation as a fumigating insecticide. Controls weevils in grain storage.

The fungus *Fusarium lateritium* has been reported to be isolated in sweetpotato sexual seed germinated after being scarified with sulfuric acid and disinfected superficially with NaOCl (sodium hypochlorite). Fungus causes chlorotic distortion in the sweetpotato leaves and has been found in fresh sexual seed and in seed stored for many years.

Determining the number of seeds

In a genebank, it is important to determine the number of seeds in each accession in the collection in order to keep an updated record of the seeds in stock. Seed counting is usually done by hand, when there are few seeds. When the seed lots are large, there are several methods to estimate the number in a seed lot. Those most used are:

1. **Proportion of count to volume**, which consists in counting seed lots of 100, 500, and 1,000 seeds, depending on the number of available seeds of the same species or accession to be counted. These seeds are then placed in glass tubes big enough to hold the seed lot, and then a line is drawn with a waterproof marker at the level, which each seed lot reaches. These tubes are then used as tares to determine the seed number in seed lots that have the same seed size as the lot used to determine these volumes.

2. **Proportion of count to weight**, which is a relation between the weight of determined number of seeds (usually 100) from an accession with an abundant number of seeds, and then on the basis of the weight of this sample, the quantity of seeds in the whole seed lot is estimated. For this purpose, all the seeds of the accession are weighed, and then 100 seeds are counted and weighed. The formula to determine the number of seeds is:

   SN= (TW/WS)*100

   SN: Seed number

   TW: Total seed weight

   WS: Weight of sample (usually 100 seeds)

3. **Count using counting machines**, which is generally used for large seed numbers. The machines, usually digital counters, are previously calibrated to register precision counting for each size of seed. It is very important to clean the machine when a new accession is processed to avoid seed mixture from one seed lot to another.

Sweetpotato seed drying at 5% of moisture content

CIP has developed a simple drying method for the storage of sweetpotato sexual seeds under medium and long-term conditions. This method allows the moisture content of the seed to be reduced to about 5%. This is a seed moisture content that is recommended for long term storage of orthodox seeds.

The seed and silica gel, when it is completely dry, with a humidity colorimetric marker (blue when dry and red when humid), are weighed in an analytical balance with 3 decimals of approximation, considering a weight relationship of 1 g of seed to 2 g of silica gel. Then the seed and silica gel are placed in separate and uncovered containers (petri dishes, for example) inside a drying bell or a plastic tray completely covered with a transparent plastic bag, which is sealed once it is filled up with samples. If plastic trays are used, the plastic bag should be sealed with a masking tape. If the room temperature exceeds 20°C, or is lower than 15°C, the plastic trays are placed inside a refrigerator or incubator for 2 weeks, with temperature controlled at 17°C.

It is very important to keep the seeds separated from the silica gel to facilitate their removal when the silica gel changes color from dark blue to reddish, by absorbing moisture and becoming humid. During the 14 days of the treatment, humid silica is replaced by a dried batch of the same weight.

When silica gel is not available, rice dried at high temperature can be used as a desiccator. Good results can be obtained by using at least 3 changes of dried rice in the two weeks of treatment.

Package sealing and storing

Once the seeds have been counted and dried, they are placed inside aluminum foil pouches which contain aluminum foil between two polyethylene sheets (PET 12µ, AL, 9µ, PE 70µ) to make it water proof. Then, these seed packages are sealed by heating. Metal or glass containers that can be hermetically sealed can also be used.

Once seeds are in packages, they can be stored in refrigerated chambers with temperatures under 4°C for short or medium storage conditions. For long term conservation, seed packages are stored in freezers at -10 to -20°C.

Seed germination tests

The sweetpotato sexual seed has a thick, very hard and impermeable testa, which makes seed germination difficult and requires scarification. There are several methods, both chemical and physical -

mechanical, that make the hard testa seed permeable and facilitate its germination.

1. **Chemical scarification with sulfuric acid**, which consists in placing the seeds in plastic sieves, and immersing them in a suitable glass containing concentrated sulfuric acid (90% approximately) for 20 minutes. The treated seeds are then rinsed in running water during the whole night to completely eliminate acid residues.

   This method has the advantage of treating large numbers of seeds, and those that do not germinate can be scarified again. The disadvantage is that there is not 100% of seed germination after this treatment. This scarification method must be executed with a lot of caution by qualified staff, because of the sulfuric acid toxicity.

2. **Scarification by wasting the seed testa with sandpaper**, which is carried out after disinfecting the seeds for five minutes in three solutions against fungi and bacteria. The first solution is hydrochloric acid (HCI) 1 N; the second is soapy water and the third is an ethanol solution at 96%. Sweetpotato seeds have a groove called hilium and the plantlet emerges from one of its extremes. The scarification of the testa consists in rubbing the opposite side to the hilium with sandpaper for wood of medium thickness in order to wear that side of the testa until it is very thin.

   If these treated seeds are going to be put to germinate, they should be placed on filter paper which is then soaked with sterile water, or placed in a plastic net in a container with sterile water for 6 to 8 hours. After this time, the seeds will be swollen and the radicle will emerge.

3. **Breaking the opposite side to the hilium of the seed using a scalpel**, which is the physical-mechanical method most used and involves making a little cut with a sterile scalpel in the opposite side to the hilium to avoid damaging the embryo. This method allows the scarification of 100% of the seeds and it is also non-toxic. However, it demands a lot of man/hours because it implies treating each seed individually.

It has been reported that sweetpotato seeds are viable for more than 20 years when stored at 18°C and 45-50% of relative humidity. The viability of sweetpotato seeds stored in genebanks is determined by in vitro seed germination tests. This is done by placing 100 seeds, depending on the number of seeds in stock of recently scarified seed in petri dishes with filter paper moistened with distilled water. The petri dishes are then placed in a germinator, refrigerator, or incubator at a constant temperature of 18°C. The evaluation is made by daily

counts of germinated seeds after 10 days, and then at 14 and 21 days from when the germination test began. The number of germinated seeds will be the percentage of germination in that seed lot. The seeds that do not germinate after 21 days are probably non-viable or dead.

Seed multiplication

Seed multiplication is done when the seed number or their germinability is under the minimun levels. In the case of *Ipomoea batatas* accessions, seed is usually obtained from the clonal material maintained in the genebank. For wild *Ipomoea* accessions, a seed sample of about 30 seeds is used for multiplication.

Since the main objective of a genebank is to maintain, insofar as possible, the original genetic variability of the samples, this is accomplished by:

1. Incrementing seed as little as possible.

2. Using appropriate techniques for long term storage, to ensure that seed viability decreases as little as possible.

3. Using 30 plants at least per accession for seed multiplication.

4. Pollinating all plants of each accession with a pollen mixture from several cultivars of the same geographical area. If seed of wild species is being multiplied, intercrossing is made between different pairs of genotypes of the same accession.

5. Preparing seed lots for the multiplication of each wild *Ipomoea* accession, in such a way that each female-plant contributes with approximately the same seed number. In the case of sweetpotato cultivars, seeds produced by all the plants in each accession are collected.

The number of seeds produced will depend on the flowering intensity, which in turn depends on the ecological niche where seed multiplication takes place, its latitude and its photoperiod. Besides, it will also depend on the sexual compatibility of the genotypes used in the multiplication. This sexual compatibility also depends on biotic factors such as the sporophytic incompatibility system at the level of the stigma, through the stile, and at the ovary level.

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Section 3.1

Farmer participation in the selection of new sweetpotato varieties

Cristina Fonseca, Juan P. Molina, Edward E. Carey

(reproduced from CIP Research Guide 5)

Introduction and objectives

The cultivation of sweetpotatoes is becoming more and more important with each passing day, and the crop has great potential as a food product. It needs a few inputs and has potentially high yields, which make it an inexpensive food. In addition, it is well suited for processing into food products for people and animals alike.

The principal goal of research and development programs is to increase not only the size of sweetpotato production areas, but also the productivity of areas where sweetpotato is already under

cultivation. Careful selection of new varieties, which show greater adaptability and stability as well as excellent agronomic characteristics-those required by farmers and consumers-will help to put that goal within reach.

One of the most important steps in developing technology is the evaluation and selection of improved or native clones for different production zones. A research methodology based on farmer participation i.e., in which farmers are actively involved in the selection of materials-could guarantee the rapid transfer and adoption of any new technology.

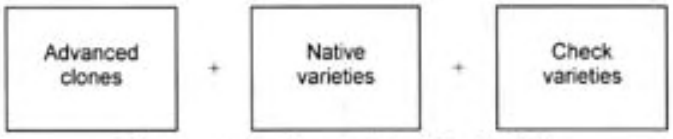
This section outlines the methods for evaluating and selecting advanced sweetpotato clones with farmer participation. It is the result of work carried out in the valleys of Cañete, Arequipa, and Piura in Peru. The results of this work have been most promising, culminating in the release of new varieties that display characteristics required by farmers.

Objectives

1. To provide researchers with guidelines for identifying and evaluating sweetpotato clones that farmers will adopt because the clones have characteristics desired by farmers.
2. To encourage farmers to participate in the selection of sweetpotato clones.

Experimental materials

Experimental materials can be made up of promising germplasm consisting of advanced clones generated by breeding programs, and of native varieties that offer some comparative advantages. These materials must also include check varieties that provide a point of reference from which to evaluate the behavior of new materials.



Components of experimental materials

Advanced clones are selected from a large number of improved clones developed by plant breeders in research programs. These advanced clones generally have excellent agronomic characteristics and such good quality that their inclusion in regional trials throughout a given country is justified.

Native varieties are those that are grown traditionally, and are the product of many years of evaluation and selection carried out by

farmers themselves. They are widespread in areas where sweetpotato is a subsistence crop. Native varieties can be obtained from germplasm banks, or from farmers' fields.

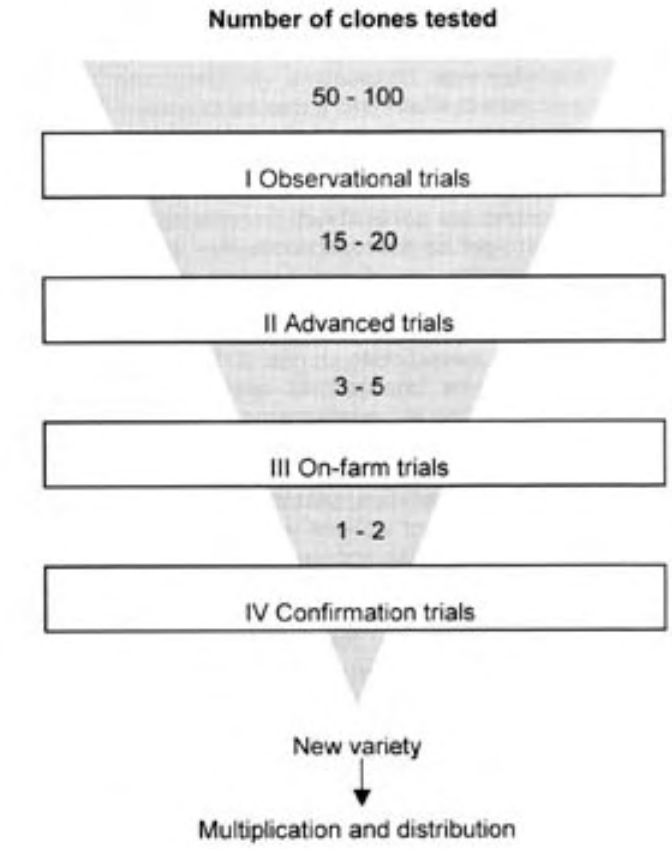
Check varieties are those sweetpotato varieties that are widely grown and are generally of commercial importance in the study area. They might be improved or native varieties, and are used for comparison with the experimental clones included in the regional trials.

It is advisable to retain the experimental codes or original names of the sweetpotato clones during the different evaluation stages. These can be changed to appropriate variety names if and when the varieties are released.

To start up regional trials with improved sweetpotato clones and native varieties, you can request propagation materials (cuttings) at experiment stations where sweetpotato breeding work is being done. You can also approach the International Potato Center (CIP).

Trials

Evaluation and selection of advanced sweetpotato clones and native sweetpotato varieties are made in the following four stages: 1) *observational trials*; 2) *statistically replicated advanced trials*; 3) *on-farm trials and multiplication of materials*; and 4) *confirmation trials for the release of new varieties*. Initially, the evaluation encompasses a large number of materials, which is then reduced as selections are made, until finally only the 1 or 2 most promising materials remain, as illustrated below:



Evaluation and selection stages for advanced sweetpotato clones and native sweetpotato varieties.

Evaluation and selection stages for advanced sweetpotato clones and native sweetpotato varieties.

We recommend that these trials be repeated during different growing seasons and under varying climatic conditions.

Observational trials

In this stage of trials, we study the agronomic performance of a large number of clones from: breeding programs (advanced clones) and germplasm banks or local collections (native and improved varieties). These clones are distributed and then planted in small plots, without replications (Table1).

Observational trials are carried out only in strategic locations representative of sweetpotato production areas or potentially promising agroecological zones.

Besides researchers, evaluations might involve a group of farmers who can help select clones to include in the advanced trials.

Table 1. Basic data for carrying out evaluation trials of sweetpotato clones.

Data	Observational trial	Advanced trial	On-farm trial	Confirmation trial
Location	Exp. Station	Exp. station/farm	Farm	Farm
No. of clones	50-100	10-20	2-5	1-3
Cuttings/clone	20-40	60	100-500	6,000-14,000
Length of row (m)*	3-5	5-10	20-100	50-100
No. of rows	1-2	3-4	3-5	20-30
Exp. design	None	RCBD**	None	Unrepl.
Replications	---	3-4	3-5	4-5
Person in charge	Researcher	Researcher	Farmer	R/F
Evaluator	F/R	F/R	Farmer	F/R

* Planting density used in this table is 0.18 m^2 per plant (0.9 m between rows and 0.2 m between plants).

** RCBD = randomized complete block design.

Advanced trials

The objective of advanced trials is to reconfirm the performance of the clones selected through the observational trials. These trials are to be considered "regional trials" and should therefore be carried out in different sweetpotato production areas. The clones are planted using a randomized complete block design with at least three replications (Table 1).

At this stage, farmer participation in the selection of clones is very important. Trials should thus take place in sweetpotato production areas, at experiment stations, or on farms (Photo 1).

Clones selected by farmers at this stage will become part of later on-farm trials.

On-farm trials

These trials are carried out on farms, preferably in collaboration with national agricultural research and extension programs or non-governmental organizations (NGO's).

Each trial consists of a maximum of five clones originally selected from the advanced trials. Because these trials are carried out by farmers, they themselves decide where the clones should be planted, and how they should be cared for and evaluated. Nonetheless, it is important to make sure that these trials are planted in the farmers' sweetpotato fields, so that the trials will not receive special treatment, and clones will be compared with the farmers' own varieties.

It is also important at this stage to multiply planting materials of selected clones by gradual increases in farmer's fields. At the same time, researchers should also multiply materials, using rapid propagation techniques, so that at least one hectare is planted with each selected clone. These materials will then be used in the final stage of confirmation trials.

Confirmation trials

In this final stage, the economic advantages of the best clones (selected during the previous stage) are evaluated and compared with those of the check varieties.

These trials are also carried out in farmers' fields, with the researcher supervising. It is recommended that at least four replications of these trials be done per production area. Each replication should cover a semi-commercial-sized plot (1000 to 2500 m^2), so that a close approximation can be reached of the productivity and economic value of the potential new variety. Table 1 lists other characteristics of these trials.

The results of the confirmation trials will help determine whether all the criteria required for the release of a new variety have been met. For further information, please check with researchers of national programs, who are in charge of releasing new varieties.

After the confirmation trials, the next logical step is to multiply and distribute planting materials of the new variety. These materials should be distributed to farmers as well as institutions interested in sweetpotato cultivation.

Figure 1. The planting of an advanced trial of sweetpotatoes in an Andean valley (Cajamarca, Peru) with participation by local researchers and farmers.

Evaluations

In the different trials to evaluate and select sweetpotato clones, two types of evaluations are carried out: technical and participatory. Researchers and farmers should actively collaborate during these trials. The evaluations have the following characteristics:

	Technical evaluation	+	Participatory evaluation
Person in charge:	Research team		Farmers
Variable	Quantitative		Qualitative
Data	Measurements		Interviews

Types of evaluation and their characteristics

Technical evaluation

For this evaluation, agronomists use a predetermined set of variables in the observational and advanced trials (Table 2), so that they can compare the behavior of advanced clones at different sites.

The evaluation variables generally apply only to the storage root. This technical evaluation takes place four to five months after planting, so that early varieties can be identified. The actual moment of evaluation, however, is completely dependent on the agroecological and socioeconomic characteristics of the production area. During this evaluation, samples of storage roots of each of the trial clones are also taken to determine dry matter content (Photo 2).

The evaluator should take note of the following basic data: planting date, planting density, number of cuttings transplanted, date of harvest, trial management data (fertilization, irrigation, weeding, etc.).

Table 2. Variables per evaluation type carried out during the selection of clones.

Variable	Evaluation type	Scale*
Harvested plants	number	
Plants with storage roots	number	
Skin color	visual rating	1-9
Flesh color	visual rating	1-9
Pest damage	visual rating	1-9
Cracking	visual rating	1-9
General evaluation	visual rating	1-9
Marketable roots	number and weight	
Non-marketable roots	number and weight	
Weight of foliage	weight	

* See Appendix 1 for details.

Figure 2. Technical evaluation: counting and weighing roots of sweetpotato clones.

Participatory evaluation

For this evaluation, the participatory research methodology (Ashby et al., 1989) is used, in which three basic elements come together:

Elements of participatory research

This evaluation can be included in every type of trial, and must be adapted to the conditions characteristic of each trial zone. The farmers (men and women) who participate in the evaluation carry out two types of tests: taste tests and agronomic evaluations.

A taste test is undertaken to determine the culinary quality of the sweetpotato variety. To this end, two or three roots per clone to be tested are taken from the experimental field, placed in net bags, and identified with symbols (letters or numbers) written on identification tags in indelible ink or felt-tipped marker. The roots are then cooked, so that the participants can taste small pieces of each clone. Their assessment can be recorded on a chart, which might look something like the example below:

Clone	Appearance			Taste		
	☺	😐	☹	☺	😐	☹
A	X				X	
B		X				X
C			X			X
Z	X			X		

Chart for taste trial: Example of results

After the participants have tastes one clone, they should take a sip of water, so that they will have less trouble making a distinction between the taste of that variety and the next.

The agronomic test is done to determine the commercial quality and productivity of storage roots. Each evaluated clone should be identified, preferably with symbols (letters or numbers) written on plot markers.

For this test, it is recommended that groups of two to four farmers be formed, so as to encourage discussion and comparison of their evaluations. Their most relevant observations about the clones can be recorded as follows:

Clone	Preferred	Rejected	Why?
A	X		High yield
B	X		Even shape
C		X	Pale skin
D	X		Good looking
E		X	-----

Chart for agronomic test: Example of results

At the end of the evaluations, the participants decide by consensus which clones should be selected. This meeting is also useful for generating discussion on the problems that farmers face in the cultivation of sweetpotato. These problems can then be addressed by local researchers.

The technique used to acquire comments and contributions for the selection of clones can be the "informal interview" (Rhoades, 1989), during which simple questions such as the following can be asked:

- What differences are there between the clones?
- What advantages or disadvantages do you see?
- Which clones are good, and which are bad?
- Others.

Photo 3. Farmers and researchers evaluating advanced sweetpotato clones in an observational trial.

Supplies needed during the trials are:

- For the participatory evaluation:
  - Forms for both the taste and agronomic trial.
  - Identification labels for the clones.
  - Pencils for the farmers.
  - Notebooks.

- For the technical evaluation:
  - Forms for recording information on evaluation variables (Appendix 2).
  - Labels for identifying the cuttings of the clones to be propagated.
  - Nets or bags for storage of roots.
  - Appropriate field scale.

Conducting evaluations

Observational trials

These trials are carried out using a large number of clones, and therefore require a lot of time and attention. Participation by a group of farmers at these trials, however, will make them more objectives.

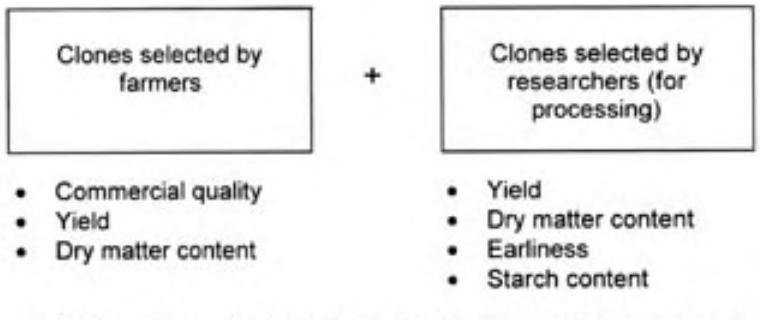
Participatory evaluation

Farmer presence and participation are deemed indispensable during the agronomic test. The ratio of number of clones to number of farmers will determine how many clones are evaluated per group of 2-4 farmers. The clones that each group selects should be marked with a label.

After these preliminary selections have been completed, all the farmers gather together to evaluate the selected clones, and to choose the most promising ones, which will then be used in the next stage, the advanced trials.

Technical evaluation

Once the farmer evaluation has been completed, the selected clones undergo the technical evaluation. The research team makes selections based on the clones' potential for industrial use, identifying materials that have high yield and good skin and flesh color. This evaluation uses technical variables (outlined in Table 2 and Appendix 2). These varieties are later used to evaluate other characteristics, as shown below:



Selection of sweetpotato clones by farmers and researchers

Advanced trials

Advanced trials evaluate a smaller number of clones, which are planted using a randomized complete block design. At this stage, participation by a group of farmers is of utmost importance.

Participatory evaluation

The participatory evaluation is used in much the same way as in the previous trial. The only difference is that, besides the agronomic test, a taste test can also be carried out.

Taste test (Photo 4)

This is carried out in the experimental plot. Cooked storage roots, each carefully labeled, are placed on a table or board for farmers to taste. It is advisable to carry out this test at the beginning of the evaluation, before farmers have selected their preferred varieties and allow their judgment to be influenced by that choice.

Photo 4. Farmers conducting a taste test with sweetpotato clones

Agronomic test

This can easily be adapted to the block design, in which varieties are arranged into three replications, as shown below:

Block I	Block II	Block III
All the plants in a row are harvested to show total production of roots.	The storage roots are divided into two groups: marketable and non-marketable.	The plants are not harvested, to observe and evaluate foliage.

To evaluate these blocks or replications, farmers record on a chart the most important observations about each clone, whether it be preferred or rejected (Photo 5). For this purpose they use their own selection criteria (Appendix 3).

Photo 5. Agronomic test: farmers evaluating sweetpotato clones.

Finally, the clones selected by each group are identified. The clones that will be evaluated by the entire group of farmers are carefully labeled (Photo 6).

Figure 6. A group of farmers selecting promising sweetpotato clones.

Some farmers will probably wish to take the selected clones to their own fields. This is a good moment to make their acquaintance, in order to include them in the on-farm trials.

The technical evaluation is carried out on all the clones in the trial, using the technical variables (Table 2 and Appendix 2). After this evaluation has been completed, samples of the storage roots are taken to determine dry matter content.

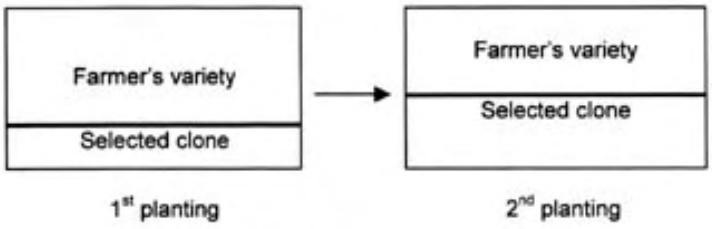
The data are statistically analyzed and differences among means are analyzed (LSD) to identify the clones that yield the most fresh matter and dry matter. Determination of dry matter content - as well as starch content and quality - is important for clones destined for industrial use.

Clones with potential industrial value will continue to be evaluated at an experiment station, to determine their industrial use or their appropriateness for human or animal consumption. The selected materials will be included later in on-farm trials and confirmation trials.

The decision to evaluate materials for industrial use will depend on whether the sweetpotato in production areas already has, or will acquire, industrial importance.

On-farm trials

In these trials, farmers evaluate the clones selected from the advanced trials. Each trial clone is planted next to the farmers' own sweetpotato variety, so that they can easily be compared. If the clones satisfy the farmer's requirements, the area in which they are planted will be increased repeatedly in the farmer's field or elsewhere:



The selected clone in the farmer's sweetpotato field

It is recommended that neighboring farmers be invited to the harvest, so as to promote discussion and comparison of the clone's advantages (Photo 7).

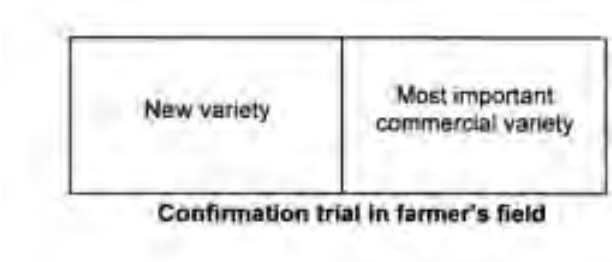
Figure 7. On-farm trials: farmer harvesting sweetpotato in her field to evaluate its characteristics.

The research team will carry out the follow-up for these trials. The information gathered from the farmers is important for helping to determine which clones will become part of the confirmation trials. The researcher should carry a notebook in which to record the following data about the follow-up:

- Farmer's name
- Location of the field
- Names of the clones planted
- Number of plants or area planted per clone
- Dates the clones were planted and harvested
- Yield

Confirmation trials

This is the most advanced stage in the selection procedure for new varieties. At this stage, yield and economic profitability are measured and compared with those of the most important local variety. The field distribution can be as follows:



The two most important parties in this evaluation are the local researchers, especially extensionists, and the farmers, who will compare the advantages and disadvantages of the new variety with those of the local variety.

This is also a good way to spread the new variety to areas where sweetpotato selection trials are being conducted.

In this last stage, the technical evaluation must also be done, using variables outlined by national institutes to assess the productivity and profitability of the new variety.

Once this variety has been approved for release by the appropriate institutions, the research team will begin extensive multiplication of the seed in experimental or farmers' fields, so that it can provide interested farmers with planting material of the new variety.

Other trials

Determination of dry matter content

The percentage of dry matter in the storage roots is measured for clones selected during observational trials as well as for all clones in the advanced trials.

The following methods are used:

a. Oven method (direct method).

b. Specific gravity method (indirect method

Both evaluations must be made within 24 hours after the materials have been harvested.

a. Oven method: For this method, you must have a controlled temperature oven and a scale with a precision of 0.1 g the procedure is as follows:

a.1 Take 3-5 storage roots and cut off their ends, leaving only the midsections of medium size. Cut the sections into small pieces, for a total weight of 200 g.

a.2 Place the pieces in the oven and dry at 60° C for about 72 hours, until the weight stabilizes.

a.3 Weigh the dried sample and record the weight.

a.4 The percentage of dry matter is calculated as follows:

$$\%DM = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

b. **Specific gravity method:** This method allows a quick estimate to be made of the relative amount of dry matter in the trial clones. It is best to use a precision scale (for example, an HOMS Model 500 g x 5 g Instrument Laboratory Scale). The scale should be tared and hung on a tripod over a container of water (a bin or a large bucket). Net bags made of nylon, with neutral buoyancy, should be used to hold the roots.

The procedure is as follows:

- Weigh 3 or 4 kg of sweetpotato storage roots in a net bag. Record the weight in grams.
- Weigh the same roots in their net bag while they are submerged in water. Record their weight while in the water.

$$SG = \frac{\text{Weight in air}}{\text{Weight in air - weight in water}}$$

Post-harvest trials

Post-harvest trials are conducted for clones selected from the advanced trials and the confirmation trials, so as to determine the resistance of the roots to packing, transport, and storage.

Packing trials: The process of packing is simulated so that the amount of damage suffered by the storage roots during the process can be evaluated.

Transport trials: The bags of storage roots are dropped from varying heights, simulating the treatment the roots receive when they are moved from the field to the transport vehicles. The resistance of the roots to bumping and friction is evaluated.

Storage trials: These are carried out to measure the amount of time that roots can actually be stored. Three factors are important: 1) loss of weight, 2) amount of rotting, and 3) amount of sprouting. These evaluations can be measured on a scale of 1 to 9, from very poor to excellent.

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Appendices

Appendix 1. Rating criteria and scales for storage roots*.

Skin color

1 White*

2 Cream

3 Yellow

4 Orange

5 Brownish-orange

6 Pink

7 Red

8 Reddish-purple

9 Purple

Flesh color*

1 White

2 Cream

3 Dark cream

4 Pale yellow

5 Dark yellow

6 Pale orange

7 Medium orange

8 Dark orange

9 Strongly colored with anthocyanins

Pest damage and rotting

1 No damage

5 Moderately damaged

9 Totally damaged

Numbers in between indicate intermediate degrees of damage or rotting.

Cracking

1 None

5 Moderate cracking

9 Heavy cracking

Numbers in between indicate intermediate degrees of cracking

General evaluation

1 Very poor

5 Average

9 Excellent

Numbers in between indicate intermediate degrees of overall appearance of the roots (skin and flesh color, form, size, surface, yield, uniformity, among others).

*Huamán 1988.

Appendix 2. Variables in sweetpotato clone evaluation.

Block	Clone No.	Clone name	No. of cuttings planted	No. of plants harvested	No. of plants with storage roots	No. of roots		Weight of roots		Root color		Pest damage	Rotting	Cracking	General evaluation
						Marketable	Non-marketable	Marketable	Non-marketable	Skin	Flesh				

Appendix 3. Description of selection criteria for sweetpotato clones used by farmers in Cañete, Peru.

	Category		
Criteria	**Accepted**	**Rejected**	**Explanation**
	Criteria for storage roots		
Commercial quality			
Skin color	Purple, orange, yellow, and red	White or cream	Marketable quality
Flesh color	Yellow-orange	White or purple	Marketable quality
Form	Round, fusiform, oval	Elongated, deformed	Marketable quality and transport
Surface	Smooth	Ribbed, veined	Marketable quality
Pest damage	None, slight	Moderate to heavy	Marketable quality (*Eucepes* sp. and *Botrynus* sp.)
Production			
Yield (# of roots/plant)	3-6 roots/plant	Less than 3 roots/plant	Yield and profitability
Weight	150-300 g	< 150 g and > 300 g	Yield and quality
Uniformity of size	Uniform	Not uniform	Yield and quality
Earliness	4-6 months	< 6 months	Yield and crop rotation
Transport			
Skin thickness	Thick	Thin	Resistant to transport
Sprouting	Absent	Present	Marketable quality
	Criteria for foliage		
Leaf			
Pubescence	Absent or glabrous	Hairy, abundant	Quality
Amount of foliage	High	Low	Quality
Leaf area	Entire leaves	Lobed leaves	Quality
Color	Dark green	Light green	Quality
Stem			
Consistency	Succulent	Dry and brittle	Quality
Length	1-2 m	Less than 1 m	Quality
Pubescence	Glabrous	Hairy	Quality

Source: Fonseca *et al*., 1992.

Section 3.2

Procedures for the evaluation of pathogen-tested sweetpotato clones

Edward E. Carey and Daniel M. Reynoso

Introduction

The selection of new varieties is an important aspect of sweetpotato crop improvement. New varieties can provide farmers with improved yields, earliness, control of diseases and pests, and quality characteristics, at little or no additional cost. Those interested in the selection of new varieties include agricultural researchers, development and extension workers and, of course, farmers.

The starting material for a sweetpotato variety selection program may be either sexual seed or previously existing clones. Sweetpotato is a vegetatively (also called clonally or asexually) propagated crop, but new varieties come principally from seeds produced by cross-

pollination. While large populations of seeds are the starting material used by established breeding programs, the process of their initial evaluation takes longer and is thus more expensive than the evaluation and selection of previously existing clones. Previously existing clones are the logical first step for evaluation and selection by newly-established variety selection programs, and are also valuable to established breeding programs as a source of potential new parental material and varieties.

Several sources of previously existing clonal germplasm are available for testing, including experimental clones and varieties released from breeding programs, as well as farmer-selected landrace varieties. Within a country, sources of clones for testing may include breeding programs, germplasm collections (gene banks), and farmers, or clones may be obtained internationally. CIP maintains a large collection of pathogen-tested sweetpotato clones available for international distribution and testing (CIP, 1996). This collection includes important landrace and released varieties from many countries, and elite experimental clones from leading sweetpotato breeding programs around the world.

The decision on which source(s) of clonal germplasm to use and how to proceed with their evaluation should be based on an understanding of current and previous sweetpotato varietal selection efforts in your target area.

This document outlines procedures recommended for the evaluation of CIP pathogen-tested clones. It provides data collection forms for initial field evaluations, and makes suggestions for data processing and evaluation. Recipients of sweetpotato germplasm from CIP are requested to use these forms to provide us with information on the performance of pathogen-tested sweetpotato clones.

Procedures

Multiply clones for trial and verify their identities

Pathogen-tested sweetpotato clones may be distributed internationally as in vitro plantlets, or in some cases, as cuttings. In vitro plantlets are delicate and require special care when being transferred to in vivo. Procedures for the transfer of in vitro plantlets to in vivo are outlined by Dodds *et al.* (1991).

Upon receipt, clones should be multiplied to produce planting materials for initial trials. Locally important or standard “check” varieties with which the introduced materials will be compared should be included in multiplication plots to provide uniform planting materials for trials. You should try to ensure that planting materials for any trial come from a single source (Wilson *et al.*, 1989).

Take care to verify and maintain the identity of clones during the process of multiplication and evaluation. This can be done through careful labeling of clones and checking of identities using morphological descriptors. To guard against errors, it is a good idea to identify CIP clones using both names and CIP numbers, because errors often occur during labeling and it is more difficult to detect numerical errors than spelling errors. For example, 440027 can easily become 420027, but there is no possibility of confusing the names - Ning Shu 1 and Zapallo - of the two clones.

The identity of clones in multiplication plots should be confirmed by comparing the appearance of each clone against the morphological characteristics on CIP's pathogen-tested list (CIP, 1996). This comparison should be done with the realization that the environment influences several of the morphological descriptors of sweetpotato, particularly with respect to the intensity of pigmentation. Therefore, you should not expect published descriptors (taken in Lima, Peru) for a particular clone to be identical to the observed descriptors for the same clone undergoing multiplication at a different location, but you should expect them to be very similar. If the published and observed descriptors, including pigmentation of foliage and roots, and especially leaf shape, coincide or are similar, you can feel comfortable that the clone is correctly identified. If published and observed descriptors vary markedly for a particular clone, a mix-up has likely occurred. In this case, the clone can still be entered in trials, but it should be renamed, so that you will not provide CIP and others with incorrect information on the performance of that pathogen-tested clone.

Conduct preliminary evaluations of adaptation and acceptability

Although some introduced clones will probably perform well in your target environment, many will probably not, due to poor adaptation to climate, soils or agronomic practices, or to susceptibility to diseases and pests. In addition, introduced clones may not have desired root or foliage quality characteristics. We therefore recommend that you initially evaluate introduced clones in observational trials with small plots, replicated once or twice, under agroecological and agronomic conditions representative of your target environment. This could mean that you should evaluate introduced clones in more than one production region (agroecology) or season. Promising clones from your initial observational trials should be selected for inclusion in more advanced trials with larger plot sizes and more replications at each location.

In many countries, sweetpotato has been an under-researched crop and variety selection criteria have not been clearly defined by researchers. Although yield and disease and pest resistance will always be important selection criteria, a number of additional factors

may be equally important. For example, in places where sweetpotato is an important food crop, taste will be important, and selection should be based on the tastes of local consumers. If the breeder-agronomist involved in variety selection is not sure of selection criteria, it is probably a good idea to ask experienced farmers for their evaluation of the introduced clones at the harvest of the adaptation trials. To accomplish this, you might evaluate introduced clones in on-farm trials, or invite farmers to your harvests on experiment stations (Fonseca *et al.*, 1993).

Incorporate clones into a routine selection scheme

If you have an ongoing breeding program, we suggest that you incorporate introduced clones directly into your standard trial scheme. Examples of sweetpotato selection schemes are provided by Hahn (1982), Martin, (1983), Jones *et al.* (1986), Wilson *et al*. (1989), Kukimura *et al.* (1990) and Saladaga *et al*. (1991).

In our sweetpotato breeding program for the lowland humid tropics at Yurimaguas, Peru, we incorporated introduced pathogen-tested clones in our routine breeding trials at an early stage of selection —the **"observational trial" (OT)**. To gain an idea of the earliness of clones, we routinely planted two blocks, which were harvested at early and later dates (normally 90 and 120 or 150 days after planting). Each clone was planted at random only once in each block, in a single-row, 10-plant plot. Trials were bordered by planting guard rows on all four sides of each block, to provide competition to all entries.

Clones selected from the OT advanced to the next stage of evaluation, the **"preliminary trial" (PT)**. At this stage, we normally used single-row, 20- to 30-plant plots with two replications, and two dates of harvest. Trials were planted in a randomized complete block design (RCBD). A point, which probably deserved greater attention, was the reduction of inter-plot competition effects, which are highest when single-row plots are used. These effects can be reduced by planting the same number of cuttings, but in shorter two-row plots, or by grouping clones according to their growth habit.

Clones selected from the PT advanced to the **"advanced trial" (AT)**. At this stage, we used three-row, 60-plant plots with three replications and one date of harvest (120 days), also planted as an RCBD. These trials were repeated over seasons. The best clones were advanced to multilocational trials, having a similar design to the ATs, and were also incorporated in our hybridization blocks.

Collect and report data

We are very interested in receiving information on the performance of CIP pathogen-tested clones. This will help us to improve our knowledge about their yield and end-use potential, range of

adaptation and reaction to pests and diseases, and to improve the targeting of future germplasm shipments. To assist with uniform collection and reporting of data on the performance of introduced clones, we have developed a set of forms, which are included in this guide. The forms provide space for filling in detailed information about the conditions under which your trials are conducted, the performance of the experimental clones in your trials, and whether they are selected. Instructions for their use are given in the next section.

Analyze data and select clones

To facilitate analysis and decision-making, raw data should be transformed into reference units of general acceptance. For instance, the number of harvested plants divided by the number of planted cuttings gives rise to survival; yield measured in kg/plot can be converted into t/ha, etc. Then, sorting, ANOVA, and mean comparisons become useful tools for clonal selection.

Description of data collection forms and instructions for their use

For most evaluations that involve rating scales, we use a scale of 1 to 9, where 1 indicates the lowest possible value of the trait being evaluated and 9 indicates the highest possible value. Thus, for reactions to diseases and pests, 1 indicates absence of the problem, and for hedonic (like/dislike) evaluations, 1 is the worst value. Instructions for rating specific traits are provided below. **Note: In some places, established breeding programs may have already developed different rating scales, for example, 1 to 5. In such cases, please use your rating scale and indicate the scale used when returning data to CIP.**

Form 1: General trial information

This form provides spaces for essential information on trials, such as location, plot size, and trial management practices.

It also provides space for the results of soil analyses and meteorological data which, if available, may help in the interpretation of trial results.

Form 2: Planting, establishment, foliage vigor, and foliar disease and pest evaluations

a) To be filled out at time of planting:

- Plot # = Plot number.
- Rep # = Replication number of plot (if trial is replicated).
- Name (CIP #) = Name of clone (and CIP number, if a CIP clone).
- # Cuttg Pltd. = Number of cuttings planted.

b) Data to be taken during the growing season, prior to harvest:

- Estab (3 wks.) = Number of cuttings established 3 weeks after planting.
- Fol. Vigor (6 wks.) = Assessment of foliage vigor using a 1 to 3 scale, where 1 is low vigor, 2 is intermediate and 3 is high foliage vigor.

Diseases and pests vary with location and season, and may not even occur in some trials. Form 2 provides spaces for up to six diseases or pest evaluations during the growing season. The name of the disease or pest evaluated should be noted at the head of the column, and the date of evaluation noted at the foot. Disease or pest reaction of each clone should be noted using a standard 1 to 9 reaction scale (see note above on rating scales).

The 'comments' column may be used for any additional observations.

Form 3: Agronomic data

a) To be filled out at time of planting:

- Plot # = Plot number.
- Rep # = Replication number of plot (if trial is replicated).
- Name (CIP #) = Name of clone (and CIP number, if a CIP clone).

b) To be taken at time of harvest:

- Wt. Tops (kg) = Fresh weight of foliage (in kg).
- # Plts. Harv. = Number of plants harvested.

- # Plts. w/out Stor. Rts. = Number of plants without storage roots.
- Gen. Eval. Stor. Rts. = General evaluation of storage roots. A subjective evaluation of the attractiveness and uniformity of the storage roots of the plot, using a 1 to 9 scale, where 1 is very poor, 9 is excellent and 2 through 8 represent increasingly favorable intermediate evaluations.
- Large Roots, # + Wt. (kg) = Total number and weight of large roots harvested.
- Small Roots, # + Wt. (kg) = Total number and weight of small roots harvested.

Root size classes should be defined by the researcher. We classify as small, roots with a diameter of less than 2.5 cm.

- Storage root rots, pests or defects (Name and score). Problems of storage root rots, pests or defects should be noted here, identifying the problem and rating it using a 1 to 3 scale of severity, where 1 is slight, 2 is moderate and 3 is severe. Common defects include sprouting, cracking and uneven shapes. Please indicate type of pest (e.g., *Cylas* weevil).

The comments column may be used for any additional observations.

Form 4: Postharvest quality evaluations

Clones with acceptable agronomic performance should be evaluated for postharvest quality traits of importance in your target region. These may include eating quality and dry matter content.

Form 4 also provides space for the evaluation of storage root skin and flesh color, which are more accurately determined in the laboratory following washing of the roots, than in the field.

Root Color - Skin or flesh may be evaluated as follows (if other scales are used, please describe them):

Root Skin Color	Root Flesh Color
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 = ark cream 4 = Pale yellow 5 = Dark yellow 6 = Pale orange 7 = Intermediate orange 8 = Dark orange 9 = Strongly pigmented with anthocyanins

Storage root dry matter content

This determination requires an oven and a balance that is accurate to 0.1 g. Our recommended procedures are described below.

1. It is desirable to carry out the initial steps of dry matter determination within 24 hours after harvest. This is to avoid postharvest changes in dry matter content prior to dry matter determination.

2. Thoroughly chop the medial sections of 3 undamaged, marketable-sized roots into small cubes. Mix thoroughly and take a 200 g sample.

3. Place the sample in an open-topped drying container, such as a paper bag, and dry at 60 °C for 72 h, or until weight is stable. Note: failure to completely dry samples will result in overestimation of dry matter content.

4. Weigh dried sample, making sure not to include the weight of the drying container, and record weight.

5. Percent Dry Matter = (dry weight/fresh weight) x 100.

Note: Dry matter content of sweetpotato tops can be determined using similar procedure to those for storage roots. However, care must be taken to process samples immediately after harvest (to avoid postharvest dehydration). Care must also be taken to obtain a representative sample, including both basal and apical vine sections for drying. To do this, several vines should be taken and thoroughly chopped, prior to taking a sample (200 g) for drying.

Eating quality

An evaluation of eating quality of boiled or steamed roots, leaves, or any other products made from the sweetpotatoes is requested, if appropriate. Several consumers should be asked to evaluate samples, and form 4 should be used to report mean results. Evaluators should be asked to give a general evaluation of appearance, taste and overall acceptability (final assessment) by answering the question "How do I like this sample?" Use a 1 to 9 scale, where 1 = very bad, 9 = excellent, and 2 through 8 represent intermediate values. For adequate evaluations of eating quality, it may be advisable to seek the assistance of a food scientist.

Form 5: Summary of clonal evaluation

Use this form to indicate whether experimental clones have been rejected, or selected, and to provide additional summary information on clones evaluated.

List clones and indicate whether the clone has been rejected (will not be evaluated again) or selected for further evaluation. Please note whether you consider that selected clones have potential for varietal release, or for use as parents in a breeding program. In addition, please use the comments column to note the main end-use purpose - table, industry, animal feeding, or other - for which you think a selected clone has potential. Important strengths or weaknesses of clones should also be noted in the comment column.

Suggestions for data analysis and clonal selection

To qualify for selection, a clone should show superiority over the currently important local check cultivars for traits of importance to farmers, traders, processors, and consumers. Because of the small plot sizes and lack of replication in observational trials, it is not appropriate to extrapolate yield data to tons per hectare. In fact, yields should not be taken into account in OTs except for a rough classification (e.g., high, medium or low-yielding clones). The inclusion of more than one check plot may be helpful for proper comparisons.

In replicated trials, it is appropriate to extrapolate to yields per hectare. A common error, which can lead to overestimated yields, is to multiply yield per plant by the theoretical number of plants per hectare. This should be avoided. Rather, yields per hectare should be estimated by extrapolating from yields per plot. It is generally inadvisable to make yield comparisons on the basis of yield per plant, particularly when the number of plants harvested per plot is variable.

A number of variables, which may be useful in evaluating the performance of clones, can be calculated from the raw data of agronomic trials. These include:

Percent establishment = (Number of cuttings established/Number of cuttings planted) x 100.

Percent survival = (Number of plants harvested/Number of cuttings established) x 100.

Percent of plants without storage roots = (Number of plants without storage roots/Number of plants harvested) x 100. A high percentage of plants without storage roots indicates lack of adaptation or lateness of a clone.

Large root yield (t/ha) = (Weight of large roots in kg/Plot area in m^2) x 10.

Small root yield (t/ha) = (Weight of small roots in kg/Plot area in m^2) x 10.

Total root yield (t/ha) = Small root yield (t/ha) + Large root yield (t/ha).

Foliage yield (t/ha) = (Weight of tops in kg/Plot Area in m^2) x 10.

Root dry matter yield (t/ha) = (Storage root percent dry matter x Total root yield)/100.

Fresh biomass yield (t/ha) = Foliage yield (t/ha) + Total root yield (t/ha).

N° of large roots per plant = Number of large roots/Number of plants harvested.

N° of small roots per plant = Number of small roots/Number of plants harvested. Large numbers of small roots may indicate potential for higher yields at later harvest dates.

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DATA COLLECTION FORMS FOR TESTING OF SWEETPOTATO CLONES

Form 1. **General trial Information**

1. Trial Code_____ Name and institute of researcher (s)_____
2. Trial location_____ Agroecological zone_____
   Latitude_____ Longitude_____ Altitude (m)_____
3. Trial type_____ Design _____ N° reps _____ Total N° clones _____ N° Checks_____
   Name (s) of check clone (s) _____
4. Date of planting _____ Date of harvest _____ Duration (days) _____ Season _____
5. Plot size: N° cuttings _____ N° rows _____ Length (m) _____ Spacing between and within rows (m)___x_____
6. Trial planted on ridges, mound, or flat? _____ Irrigation date (s) / volume (s) _____
   Fertilization_____Previous crop_____
7. Soil type _____
   Texture _____ pH _____ Organic matter % _____ CEC meq / 100 g _____
   Aluminium saturation % _____ EC mmhos / cm _____Ca CO_3 % _____
   Nutrients analysis _____
8. Meteorological data during trial (means by month or fraction of month):

Meteorological data	Month					
	1	2	3	4	5	6
Mean Temperature (°C)						
Mean Max. Temperature (°C)						
Mean Min. Temperature (°C)						
Rainfall (mm)						
Radiation (MJ/m^2)						

9. Other_____

DATA COLLECTION FORMS FOR TESTING OF SWEETPOTATO CLONES

Form 2. Planting, establishment, foliage vigor, and foliar disease and pest evaluations

Trial code: _____ Trial location _____

Date of planting: _____

Plot #	Rep #	Name (CIP #)	# Cuttg Pltd.	# Estab. (3 wks)	Fol. Vigor (6 wks)	Disease or pest evaluated. Indicate name at head of column, and date of eval. At foot						Comments
Date of Evaluation:												

DATA COLLECTION FORMS FOR TESTING OF SWEETPOTATO CLONES

Form 3. **Agronomic Data**

Trial code: _____________ Trial location ____

Date of planting: ____

Plot #	Rep #	Name (CIP #)	Wt. Tops (kg)	# Plts. Harv.	# Plts. W/out Stor. Rts.	Gen. Eval. Stor. Rts.	Large Roots		Small Roots		Storage root rots, pests and defects (Name and score)	Comments
							#	Wt. (kg)	#	Wt. (kg)		

DATA COLLECTION FORMS FOR TESTING OF SWEETPOTATO CLONES

Form 4. **Postharvest Quality Evaluations**

Trial code: _____ Trial location _____

Date of harvest: _____

Plot #	Rep #	Name (CIP #)	Storage root color		Storage root dry matter			Eating quality evaluation			Comments
			Skin	Flesh	Fresh wt. (g)	Dry wt. (g)	(%)	Appear.	Taste	Overall	
Date of evaluation:											

DATA COLLECTION FORMS FOR TESTING OF SWEETPOTATO CLONES

Form 5. **Summary of clonal evaluation**

Trial code: _____________ Trial location ____

Name (CIP #)	Rejected	Selected		Comments on potential uses, strengths and weaknesses
		Varietal	Breeding	

Section 3.3

Preliminary evaluation of earliness in the production of storage roots in a sweetpotato collection

César Aguilar and Zósimo Huamán

There are many criteria to determine earliness maturity in the production of storage roots in a sweetpotato collection. The most common are: speed of storage root bulking, speed of root latex drying, senescence of leaf tips, and profuse flowering (Yen *et al*., 1987).

One of the methods used by sweetpotato breeders to determine the earliness of a cultivar is by determining the storage root weight at 50-60 days from planting. A correlation has been reported of r=0.82 between storage root weight at 50 days and their weight at 90 days

after planting, and a negative correlation of r=0.246 between storage root weight at 50 days and at 140 days after planting (Ramanujam and Indira, 1979). Similarly, the beginning of the presence of starch in the storage roots 8 - 9 days after planting is positively associated with earliness in the formation of storage roots (Ramanujam and Indira, 1979; Indira and Kurian, 1977). In general, an early progeny is obtained when both parents are early, because earliness is controlled by the additive action of genes (Yen *et al*., 1987).

The earliness of a sweetpotato cultivar can be estimated by determining the increase or gain in the weight of the storage roots throughout the vegetative period of the crop. Those genotypes with a greater gain in storage root weight at 105 days from planting are probably earlier than those greater weight gains after 105 days. However, sweetpotato earliness is also influenced by the sanitary condition of the plants, soil moisture content, and the initiation time of storage root formation (Milthorpe, 1967; Chatterjee and Mandal, 1976). The best opportunity to harvest an early cultivar is when the commercial size storage root weight gains begin to decline.

A practical method to determine the earliness of accessions in a sweetpotato collection is by harvesting the storage roots at 60, 75, 90, 120, and 150 days to measure weight gains. For this, the higher the number of plants sampled, the better will be the estimation of the beginning of the formation of commercial size storage roots. It is considered that 30 plants per plot, is the optimum for a good estimation of the yield potential of a cultivar. However, when the sweetpotato collection has many accessions, the number of plants used should be as many as possible to have a good preliminary estimation.

For a preliminary evaluation, each plot should have as many rows of plants as the number of evaluation periods or samplings plus two outer rows used as borders. Each row may have from 6 to 10 plants, depending on the number of accessions in the collection and the size of the land available for this evaluation. In each evaluation, all storage roots produced in at least 4 intermediate plants of each row are harvested. The weight of storage roots is then determined, considering as commercial size roots those with more than 115 g each. The non-commercial size storage roots are those with weights from 50 to 114 g each, and the pencil type roots should not be considered. For each accession, the number and weight of the commercial and non-commercial size storage roots produced in a row of plants are recorded, as well as the size of the harvested area. Sometimes an accession may have a high total weight of non-commercial size storage roots; however, the estimation of earliness depends on the total weight of the commercial size roots.

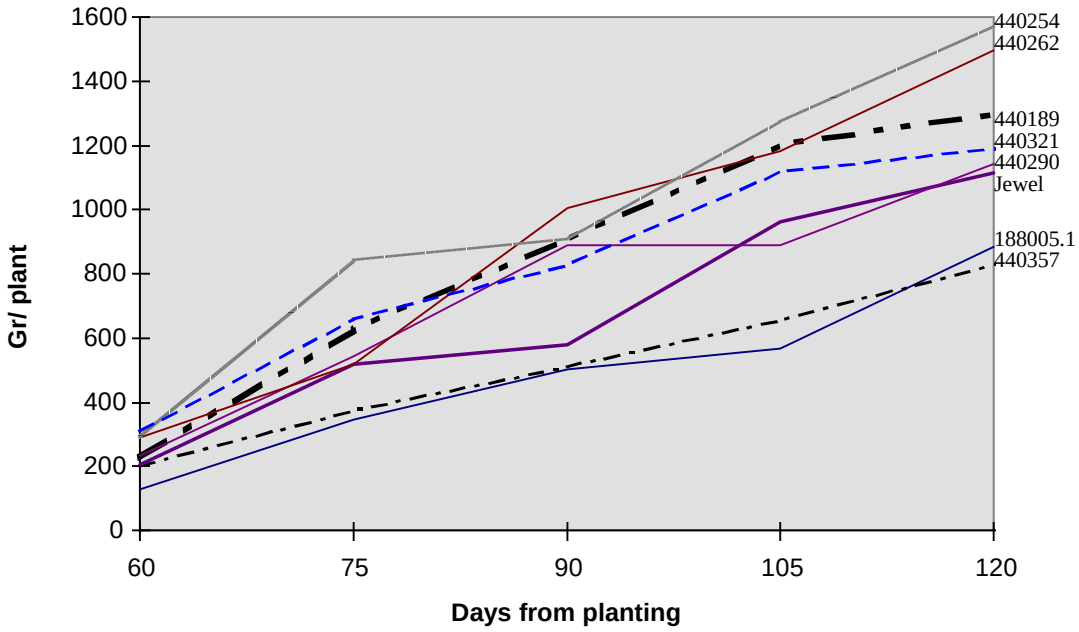

Figure 1. Storage root yield of selected sweetpotato accessions compared with that of "Jewel" obtained at different harvest periods at San Ramon, Peru.

It is a good practice to include as controls well known early maturing cultivars among the accessions to be evaluated. Similarly, it is important to repeat these evaluations in different seasons of the year in order to discard environmental effects.

Sweetpotato accessions can be classified as VERY EARLY, when they produce commercial roots in less than 90 days; EARLY, between 90 and 100 days; SEMI-EARLY, between 110 and 119 days; LATE, between 120 and 150 days and VERY LATE when they produce commercial roots more than 150 days after planting. This classification is based on the weight and the physiological maturity of the storage roots.

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Section 3.4

Methods to evaluate culinary quality and other attributes for processing sweetpotato storage roots

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Introduction

The determination of culinary quality and other attributes for processing storage roots of sweetpotato collections is very important. It allows us to estimate the quantity of nutrients and anti-nutrients contained in the storage roots, which can vary because of the environment and agronomic management of the crop.

Fresh sweetpotato storage roots contain from 16 to 40% of dry matter, of which 75 to 90% is composed of carbohydrates such as starch, sugar, cellulose, pectin, and hemicellulose (Collins and Walter, 1985), whose concentrations depend on the cultivar (Picha, 1987).

Evaluation of the culinary quality of sweetpotato storage roots

The evaluation of the culinary quality of sweetpotato storage roots is necessary to identify the preferences, and to evaluate the genetic material within a sweetpotato collection. One of the methodologies to achieve this objective is to use the sensorial evaluation technique.

The reliability of the information obtained in a sensorial evaluation depends on the people who take part in the tasting panel, or the group of tasters. In this kind of evaluation, psychological errors take place, and a brief description of them is provided below so that the evaluator may be able to eliminate them.

The habituation error, results from a tendency to continue giving the same answers when there is a group of samples in increasing or decreasing order.

The expectation error, induces the anxious taster to find a difference where there is none.

The inducement error, when the person knows how to do the test, but the containers used suggest differences where there actually are none.

The benignity error, when the evaluator is a friend of the researcher and, for that reason, gives the sample a higher assessment.

The central tendency error, when the evaluator hesitates to use the extreme values of the scale.

The contrast error, when a bad or disagreeable sample is presented after a good or agreeable one.

Order of presentation or sample position error, when different samples are assessed in the same way just because they have been presented in the same order.

The association error, when there is a tendency to repeat previous impressions.

In food evaluations, the most important errors are central tendency, position, and contrast.

The tasters who will evaluate the sweetpotato samples should be chosen from among people who have the ability to recognize differences in the attribute among the samples to be evaluated. The tasters will be asked not to eat or smoke for at least one hour before the evaluations. It is recommended not to carry out tasting sessions close to lunchtime because the tasters' appetite would make them score the samples higher than they deserve. The opposite happens if they taste after lunch. The most suitable hours for an evaluation are 10 in the morning or 2 in the afternoon, depending on the alimentary habits and customs of the evaluating group.

The attributes that can be evaluated are consistency, color, texture, and sweetness of the flesh of boiled storage roots by using the scales established in the descriptor list CIP/AVRDC/IBPGR (1991).

In order to facilitate the boiling of many storage root samples at the same time, sealed plastic bags are introduced in a big container. These bags should contain storage roots of medium size and water. Each bag should be previously identified with a number by using a marker with waterproof ink inside and outside it. As many samples as possible can be placed there as long as they are not put on top of each other. All the samples are then covered with water and boiled for a pre-established time until they are cooked.

The results are recorded in forms where each taster can write down the number of the sample evaluated and an opinion about the consistency, color, textures, and sweetness of the flesh of the boiled sweetpotatoes. These forms must have the codes used for each one of the descriptors.

Similar methodologies have been developed by Martin & Rhodes (1984), Martin & Rodríguez-Sosa (1985) and Martin (1986).

Determination of dry matter content

Dry matter content is an indicator of sweetpotato quality, and it is the raw material for the industry because it shows correlation with other nutritive characteristics.

The correlation coefficient between the dry matter and the starch content is positive, whereas its correlation with the sugar content is negative (Hamilton *et al.*, 1986).

This method is based on measuring the total weight loss of a sample kept in an oven until the weight is constant. This is done, by sampling 1 kg of sweetpotatoes of each genotype from which a sample of 100 g is taken. This sample is chopped and dried in an oven at 95°C under atmospheric pressure during five days, at the end of which a constant weight is reached.

$$\% \text{ Dry matter} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100$$

Carbohydrates

The main sugars in sweetpotato are sucrose, glucose and fructose. The first one is the most abundant in fresh roots of all the cultivars (Picha, 1985; Picha, 1987; Collins & Walter, 1985). The sucrose is a disaccharide composed of glucose and fructose without reducing power and therefore, it is not substratum of the non-enzymatic darkening. In acid conditions, it is possible to hydrolyze it in glucose and fructose by means of the invertase, obtaining the product called inverted sugar (Cheftel & Cheftel, 1976).

According to Picha (1987), glucose and fructose are the only monosaccharides found and, therefore, they are the main reducing sugars of fresh sweetpotatoes, with a slightly higher concentration of glucose.

Hamilton *et al*. (1986) reported the existence of a positive correlation between intensity of the flesh color and the content of sugars in the sweetpotato root. Thus, the white flesh sweetpotatoes show a lower sugar content than those with orange flesh. Picha (1987) also reported that from six cultivars evaluated, the four with orange flesh had more sugars than the two cultivars with white flesh.

Starch is a polymer composed of units of D-glucose with lineal bonds (alfa-1, 4) or ramified (alfa-1, 4; 1,6). The amylose and amylopectin change in absolute proportion according to the varieties and species (Fennema, 1985). The starch content in raw sweetpotato storage roots is from 33 to 73% (on a dry basis) (Truong *et al.*, 1986) and is composed of 60 to 70% of amylopectin and 30 to 40% of amylose (Walter & Hoover, 1986).

Cellulose, hemicellulose and pectin are structurally associated with the proteins and lignin (Palmer, 1982), and they constitute the fiber that is defined with ambiguity as all the non-digestible polysaccharides in the sweetpotato root (Palmer, 1982; Collins & Walter, 1985). Jones *et al*. (1980) reported that the fiber content in roots is correlated to their size; big roots show fiber levels that are significantly higher that smaller roots.

One of the reasons for reduced acceptance of the sweetpotato is the flatulence it can cause when consumed in large quantities. Several studies have been made to determine the cause of this problem. Truong *et al.* (1986) and Tsou *et al*. (1987) determined that the content of oligosaccharides in the sweetpotato is minimal. This fact suggests that the substance that causes flatulence in sweetpotatoes must be something else, probably the starch and the fiber, but not the

oligosaccharides raffinose and staquiose as happens with the legumes.

Influence of reducing sugars content in processing

The content of reducing sugars affects the degree of darkening in sweetpotato fried chips (Maillard Reaction). The higher the concentration of reducing sugars is, the darker the chips, which is the main problem in the processing (Bouwkamp, 1985; Truong & Del Rosario, 1986). The higher the concentration, the narrower is the range of frying temperatures (Bouwkamp, 1985).

Methods to determine the content of total reducing sugars and starch by spectrophotometry

Determination of the standard curve of glucose

- Weigh 1 g of anhydrous glucose and top it up to 100 ml in a volumetric flask with distilled water.
- Take aliquots from 1 to 10 ml and put them in volumetric flask glasses. Number them from 1 to 10 and top up with distilled water to 100 ml.
- From each volumetric flask glass take 1 ml of sample and put them in test tubes also numbered from 1 to 10. In tube N° 11, put 1 ml of distilled water (control).
- Add 3 ml of Ross reagent to each test tube, and place them in a boiling water bath for 6 minutes.
- Cool the tubes in ice for 3 minutes.
- Take the absorbency readings using an absorption filter of 620 nm in the equipment, which has been previously calibrated with the control.
- With the data of absorbency and glucose concentration, determine a simple linear regression and the equation for the regression line.

Determination of reducing sugars by the Ross Method

- Take 1 g of sample (freeze dried flour).
- Add 40 ml of ethanol at 80%.
- Shake it for 30 minutes.
- Filter and wash the sample with distilled water until reaching 100 ml.

- Take an aliquot from the filtered sample according to the concentration of sugars in the sample. You can start with 1 ml and add 3 ml of Ross Reactive. If the absorbency is not between 0.0969 and 0.6990 it is necessary to take different aliquots until the readings reach that range.
- Place it in water bath at 100°C for 6 minutes.
- Cool it in ice for 3 minutes.
- Read the optical density at 620 nm.

Determination of total sugars through the Ross Method

- Take 1 g of sample (freeze dried flour).
- Add 40 ml of ethanol at 80%.
- Shake it for 30 minutes.
- Filter and wash with distilled water until reaching 100 ml.
- Take 50 ml from the filtered sample and add 5 ml pure HCl.
- Place it in water bath at 65-70°C for 5 minutes.
- Neutralize it with NaOH 5N and 0.1N up to pH 6.5.
- Top it up to 100 ml.
- Take an aliquot according to the concentration of sugars in the sample - you can start with 1 ml - and add 3 ml of Ross Reactive. If absorbency is not between 0.0969 and 0.6990 take different aliquots until the readings reach that range.
- Place it in water bath at 100°C during 6 minutes.
- Cool it in ice for 3 minutes.
- Read the optical density at 620 nm.

Determination of starch content through the Ross Method

- Take 1 g of the sample (freeze dried flour).
- Add 100 ml HCl at 5%.
- Place it in water bath at 100°C for 4 hours.
- Neutralize with NaOH 5N and 0.1N to pH 6.5.
- Top it up to 250 ml with distilled water.
- Filter with Whatman # 1 paper.
- Take an aliquot according to the starch concentration in the sample; you can start with 1 ml, and then add 3 ml of Ross Reactive. If the absorbency is not between 0.0969 and 0.6990 it

is necessary to take different aliquots until the readings reach this range.

- Place it in water bath at 100°C for 6 minutes.
- Cool it with ice for 3 minutes.
- Read the optical density at 620 nm.

% Starch = (% sugar by starch hydrolysis - % total sugars) x 0.9

Proteins

Walter & Catignani (1981) reported that the percentage of raw protein (N x 6.25) in the sweetpotato fresh storage roots ranges from 0.49 to 2.24% on a wet basis, which represents percentages of 1.73 to 9.14% on a dry basis. Similar values were reported by Bouwkamp *et al*. (1985), with values between 1.3 and 10%, and by Tsou *et al*. (1987), with values between 1.34 and 11.08%, and an average of 4.22% on a dry basis.

Collins & Walter (1985) indicate that the protein content is uniformly distributed in the entire sweetpotato root, and the globulin "ipomoeine" is presented in a greater quantity.

According to Walter & Catignani (1981), Tejada (1983), and Truong & Del Rosario (1983), sweetpotato protein has a good nutritive quality because it has all the essential amino acids, mainly lysine and treonine, in sufficient quantity. Truong & Del Rosario (1986) found that the limiting amino acids in sweetpotatoes are cysteine, methionine, and tryptophane.

Determination of proteins by the Bradford method (1976)

Reagents

- Biorad stain to determine the protein.
  1 part of stain + 4 parts of distilled water, and filter them afterwards in Whatman paper # 1.

- 0.5 NaOH.
  Weigh 20 g NaOH and top up to volume of 1 liter with distilled water.

- Casein standard.
  When the sample to be analyzed is freeze dried, make a stock solution of casein of 500 µg/ml, weighing 50 mg of casein and topping it up to a volume of 100 ml with 0.5N NaOH. Shake and hydrolyze it for 2.5 hours. Then, prepare the following standard solutions:

Standard Solutions	0.5 NaOH ml + aliquots of solutions	
250 µg/ml	5 ml	5 ml 500 µg/ml stock
125 µg/ml	5 ml	5 ml 250 µg/ml solution
50 µg/ml	6 ml	4 ml 125 µg/ml solution
25 µg/ml	5 ml	5 ml 50 µg/ml solution
10 µg/ml	6 ml	4 ml 25 µg/ml solution

- BSA (Bovine Serum Album) Standard.
  Use BSA as standard when extracts of proteins or enzymes are to be analyzed.

Analysis

- Weigh 15 mg of the freeze dried storage root and put it in test tubes.
- Add 5 ml 0.5N NaOH and mix.
- Hydrolyze it at room temperature for 2.5 hours.
- Take 0.4 ml of this solution and add 5 ml of Biorad reagent and mix. Do the same with the standard solutions.
- After 60 minutes read the absorbency at 595 nm.

It is important to:

a. Prepare a control sample with 0.4 ml 0.5N NaOH + 5 ml of Biorad reagent.
b. Take into consideration that detergents can interfere with the stain.
c. The content of protein in the aliquot sample must be between 10-100 µg of protein in a volume of 1 ml.

Process to obtain flour

Selection

The storage roots for analyses must be healthy, discarding those with phytosanitary problems, bruises or rots.

Washing

The storage roots are washed totally by immersion and shaking in cold tap water, and then brushed to eliminate the soil and other foreign particles attached to the skin.

Peeling

The mechanical peeling of roots is recommended, when they have the right shape and uniform size to allow the use of the abrasive peeler. The roots that do not have these characteristics are peeled manually.

Skin-residues removal

The peeled storage roots should be inspected to remove any skin residues.

Washing

The peeled roots are washed with abundant tap water to eliminate the residues of the peeling.

Cutting

The roots are cut in 1.8 mm thick chips using a slicing machine.

Treatment with bisulfide

Chips are immersed for 5 minutes in a solution of sodium bisulfide at 0.5 % which contains citric acid, until they reach a pH 2.5.

Draining

The chips are put on racks to drain the residual water.

Drying

The chips are put in a tunnel dryer at 60°C with a speed of 1.2m/s for 12 hours.

Grinding and sieving

The grinding and sieving of the chips are done according to the regulations of each country.

Packing

The final product can be packed in polythene bags.

Sweetpotato flour yield

The flour yield of each sweetpotato genotype is determined by recording the weight of the final product and the weight of the raw material used to produce it. The flour yield is calculated by:

Flour Yield = $\frac{\text{Weight of flour obtained}}{\text{Raw material weight}}$

Processing of sweetpotato chips

Talburt & Smith (1975) define frying as a process essentially of cooking and dehydration during which the starch in the cells is gelatinized and dehydrated, and much of the water in the tissue is replaced with oil. According to Picha (1986), this last effect finishes when bubling stops.

The process is as follows (Bouwkamp, 1985 and Truong and Del Rosario, 1986):

Selection, washing, and peeling of the storage roots

Same procedures as those used to obtain flour.

Cutting

Roots must be cut in chips of 1.3 mm thickness, as recommended by Picha (1986), using a slicing machine.

Selection

The chips to be selected should have a uniform size and good appearance.

Washing

The chips will be washed by immersion and shaking in cold tap water to eliminate the starch liberated at cutting time.

Draining

The chips are placed in sieves to allow drainage of the water, and then they are partially dried with towel paper.

Frying

The chips are fried in vegetable oil at 163°C for 1.5 minutes, until the bubbling stops (Picha, 1986).

Oil draining

The fried chips are placed in sieves to eliminate residual oil.

Cooling

Fried chips are cooled until they reach room temperature.

Packing

Once the final product is cold, it is placed in polyethylene bags that are sealed hermetically.

Yield of sweetpotato chips

The chip yield is determined for each sweetpotato genotype by recording the weight of the raw material used and the weight of the chips obtained. This is calculated as follows:

$$\text{Chips Yield} = \frac{\text{Weight of chips obtained}}{\text{Raw material weight}}$$

Sensorial evaluation of sweetpotato chips

Two types of evaluation may be used. One of them evaluates the overall quality of the chips, and the other is used to evaluate the degree of chip darkening caused by the frying.

A tasting panel with some previous training in this kind of tests, should do **the evaluation of overall chip quality**. The Scoring Test recommended by Mahecha (1985) seems appropriate to evaluate the overall quality of a nutritional product.

The characteristics to be analyzed are color, flavor, texture, uniformity of size and shape, and general appearance. Panel members receive a specific scoring table for each organoleptic characteristic. The table below is similar to the one used by Mahechas (1985), but it has some modifications.

Instructions:

a. Read carefully the instructions provided together with the scoring.

b. Remember that the evaluation to be made is important in order to get valuable data which should be as reliable as possible.

c. You will receive seven samples of sweetpotato chips, which you should evaluate for their general appearance, color, uniformity of shape and size, flavor and texture.

d. When more than one characteristic flavor is found in a sample, the final scoring will be the one with the lowest value.

General appearance

This factor is related to imperfections that affect the product's appearance, such as necrosis, skin loss, broken or triturated chips, and areas with calluses, cavities and discoloration.

Scoring system for chip's general appearance

Score	Description
3.1 - 4.0	Practically free of imperfections or any combination of them that may slightly affect the product appearance.
2.1 - 3.0	Maximum of 20% of units with light or dark cinnamon-colored surface, or discoloration of more than 1 cm diameter. One broken unit.
1.0 - 2.0	Maximum of 30% of units with light or dark cinnamon-colored surface, or discoloration of more than 2 cm diameter. Two broken units.

Scoring system for chip color

Score	Description
2.1 - 3.0	Light and uniform
1.1 - 2.0	Slightly discolored; not uniform
0 - 1.0	Green or dark spots.

Scoring system for chip shape and size uniformity

Score	Description
2.1 - 3.0	Slices of uniform thickness, between 1 and 2 mm with smooth rims and minimal breaking up of chips. Maximum 15% of small units, splinters and/or irregular chips.
1.1 - 2.0	Slices of non-uniform thickness, rough or curved surfaces. Maximum 30% of small chips, splinters and/or irregular chips.
0 - 1.0	Broken or wrinkled chips.

Scoring system for chip flavor

Score	Description
3.1 - 5.0	Characteristic flavor, neutral fat and slightly sweet.
1.1 - 3.0	Insipid or too sweet, slightly burnt.
0 - 1.0	Bitter, burnt, rancid.

Scoring system for chip texture

Score	Description
3.1 - 5.0	Uniform external surfaces and moderately toasted, with adequate oil quantity.
1.1 - 3.0	Slightly hard external surfaces without oil excess.
0 - 1.0	Too soft or burnt chips soaked in oil.

Sensorial evaluation of sweetpotato chips

Name:

Date:

Characteristics	Sample						
General appearance							
Color							

Size and shape							
Flavor							
Texture							

Observations: _____

_____

_____

Evaluation of the degree of chip darkening

The group of trained panelists who will evaluate the degree of chip darkening should receive the following scoring system:

Scoring system to evaluate the degree of sweetpotato chip darkening

Score	Darkening	Qualification
1	None	Excellent
2	Very little	Very good
3	Little	Good
4	Some	Regular
5	Abundant	Bad
6	Very abundant	Very bad

Phenols

Walter *et al.* (1979) reported that the phenolic content in sweetpotato storage roots is composed of chlorogenic acid and other similar compounds, as found by Rudkin and Nelson in 1947. They also report that Kojima and Uritani in 1973 found that chlorogenic and isochlorogenic acids are phenols that appear in larger quantities.

The darkening has also been related to the concentration of phenolic compounds in storage roots which produce a green or gray coloration in some sweetpotato varieties, mainly of white flesh and high phenol content (Bouwkamp, 1985).

The measurement of the effect of oxidation agents when roots are cut is made by a visual evaluation of the darkening, using a scale ranging

from 1 (non-evident darkening) to 5 (complete darkening) (Jones, 1972).

The methodology developed by Kumagai and Yamakawa (1994) to estimate the activity of the oxidase polyphenol is recommended.

Latex and anti-nutritional substances

There is very little information about the latex content in sweetpotatoes. A Japanese researcher has identified this component as "Jalapine". It is known that its presence is an impediment for the manufacture of sweetpotato starch (Del Carpio, 1987).

According to Tsou *et al*. (1987), the presence of the trypsin inhibitor in the storage root was discovered in 1954, and it constitutes the only anti-nutritional element known in sweetpotato, because it inhibits proteolysis during digestion by reducing significantly the protein efficiency (Collins and Walter, 1985). Besides, the analyses of its activity show that it has a positive correlation with the protein content. It can be destroyed by high temperatures, and thus there will be an increase in protein digestibility (Tejada, 1983). According to Dickey *et al*. (1984), keeping the storage roots at 90°C for several minutes deactivates most trypsin inhibitors.

The relative quantity of latex can be measured in medium sized storage roots cut transversally by observing the quantity of latex eliminated and using the following scale recommended by CIP/AVRDC/IBPGR (1991).

3 Little

5 Some

7 Abundant

In vitro determination of starch digestibility

There are many methods to determine the starch digestibility, and the most recommended are those that use enzymes (Holn *et al*., 1985, 1986)

The starch of a sample is hydrolyzed with alpha-amylase and it is reduced into sugars, which are reported as maltose equivalents that are determined by spectrophotometry. The digestibility is evaluated at 5, 15, 30, 60 minutes and 24 hours of enzymatic hydrolysis.

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Section 3.5

Evaluation of potato and sweetpotato genotypes for drought resistance

Indira J. Ekanayake

(reproduced from CIP Research Guide 19)

Drought is a major environmental constraint to potato production in warm and hot tropics and for sweetpotato in the traditional cultivation areas. The problem of drought stress can be alleviated by manipulating cultural and management practices and by using adapted genotypes with drought resistance.

At CIP, we have developed a two-phased approach to screen potatoes and sweetpotatoes for their drought-resistance ability. In this publication, the methodologies used in the first phase to select for drought resistance based on rooting ability are described for wider

adoption and testing. Adapted cultivars can be selected at a country-site level since drought is extremely site-specific.

General background and definitions

What is drought?

A field definition for drought is a period without rain, of sufficient duration to cause injury to the crop and significantly reduce the economic yield. Drought begins when the readily available soil water in the root zone is exhausted (Kramer, 1983).

What is drought resistance[1]

A genotype is drought resistant when it produces an economic crop, within the limits of its production potential under conditions of limited water availability. We use this as a working definition to separate it from a more specific concept of drought resistance.

A genotype can be drought resistant due to the following mechanisms:

- Drought escape
- Drought tolerance
- Drought avoidance, and
- Drought recovery

These mechanisms are not mutually exclusive and provide the crop with the ability to resist drought at any given period during its growth cycle.

Screening methodology

Step 1: Checks

Select and use an adapted or commonly cultivated genotype as the point of reference (check clone). Also, try to use a resistant and a sensitive clone as an upper-and lower-level reference; these genotypes can be temporary references, which can be changed as more information becomes available and as more genotypes are tested.

[1] For more details, refer to CIP's Research Guide N° 30 and the reference list in the back.

Step 2: Simulated drought screening nursery

Rationale

For various reasons, at the beginning of a screening program, lack of planting materials (potato tubers or sweetpotato stem cuttings) of each genotype usually limits field experiments. The approach outlined here takes this problem into account and is designed to be tested with a minimum number of planting material of the individual test clones, i.e. 30 potato tubers or 60 sweetpotato stem cuttings.

To compensate for both the extremely site-specific nature of drought resistance and for the low test population, check clone should be planted as many times as possible (using the local variety as the check enables you to have more planting materials).

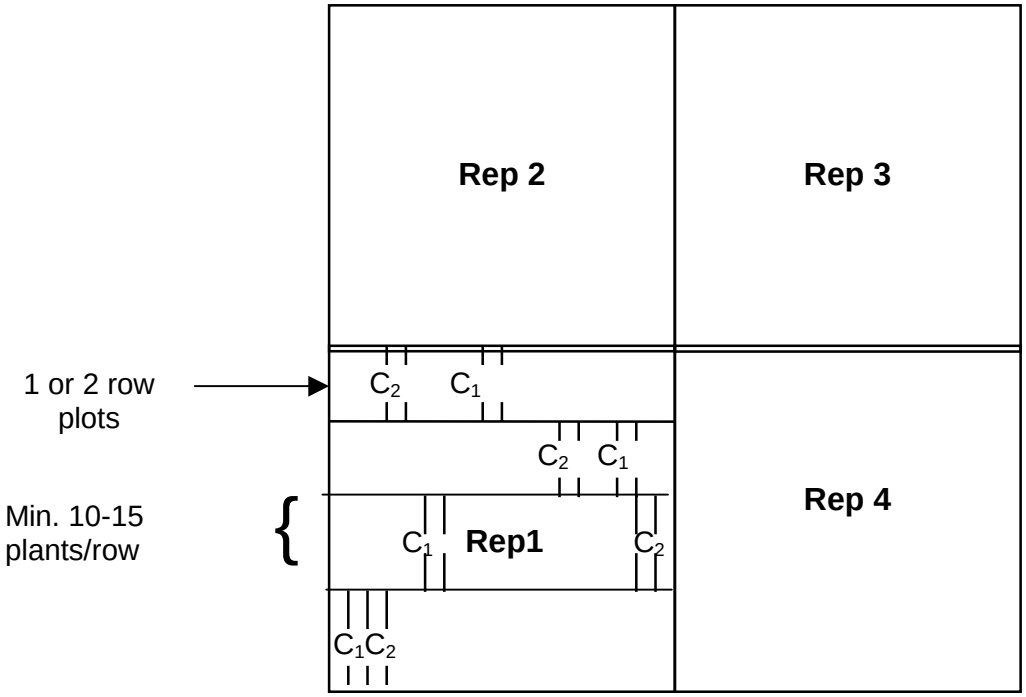
Statistical design

A randomized complete block design with genotypes as treatments is recommended (Figure 1a). For sweetpotato, a minimum of 3 replications is required; due to the large genotype x environment interaction. At least 5 replications and larger plot sizes (with more number of plants) are preferred. It can also be used a more complex but suitable design when few tubers are available for planting (Figure 1b).

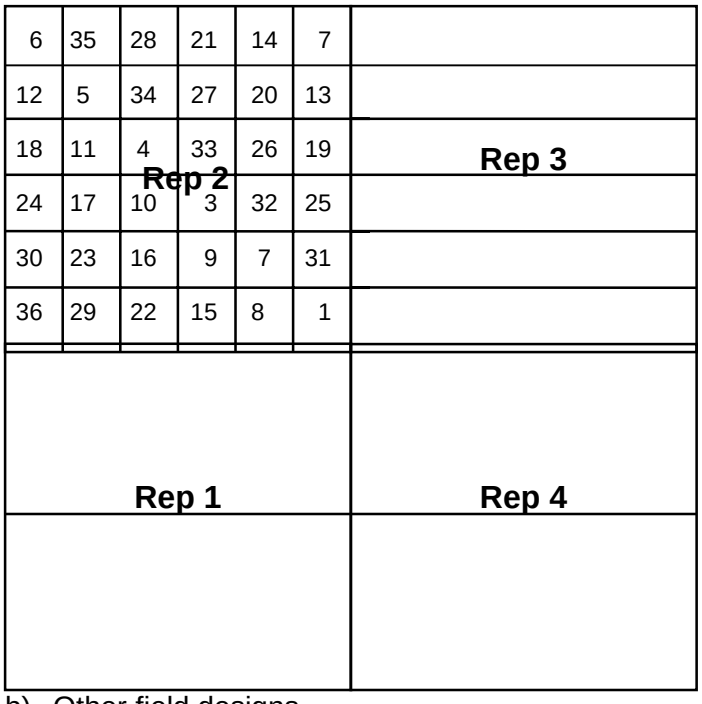
Field plot layout

Single-row plots of 10 plants each for potatoes, and two-row plots with 10 plants per row or 20 plant single-row plots for sweetpotato can be used. Plant spacing depends on the local recommendations; at CIP headquarters, plant spacing of 30 cm x 70 cm for potatoes and 30 cm x 90 cm for sweetpotato are normally used.

Figure 1. Detailed field plan with proposed guidelines.



a) Randomized complete block design.



b) Other field designs.

A single sprouted potato tuber (physiologically-young sprouted tubers are recommended) per hill or a single sweetpotato stem cutting approximately 25 cm long can be planted per hill.

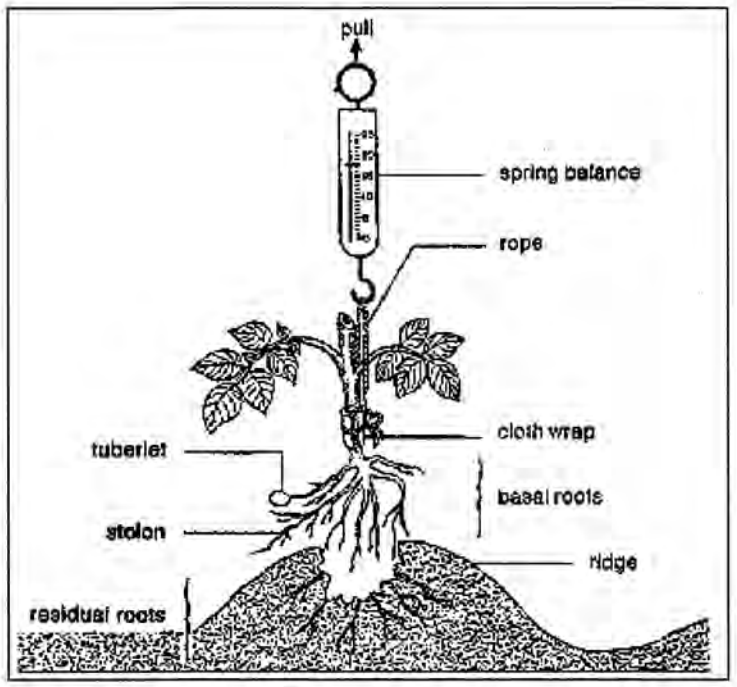
Checks should be repeated at least once (preferably more than once) in each block.

Drought simulation

Based on the priority drought period in the region, and the identified sensitive growth stage, the drought treatment can be applied to coincide with the establishment stage or tuber/tuberous root-initiation stage and then to continue until harvest. Planting can be adjusted to maximize the occurrence of predictable drought at the specific location. Our approach is to initiate the stress at approximately full canopy cover development and to expose to a continuous drought.

Intensity of drought applied can also depend on the needs of the crop and growing area: mild, moderate, or severe stress. We have used 60% of the potential evapotranspiration for a moderate drought stress treatment, or alternate irrigation cycles of the optimum water requirement to induce the field drought stress throughout the growing season. Water management practices used must be adjusted according to the total volume of water needed by the individual crop; method of irrigation (furrow, sprinkler or sub-surface); and the stage of crop development.[2]

[2] The proper uses of irrigation practices are described in CIP Research Guide N° 30, and in Haverkort, A.J. 1982. Technical Information Bulletin 15, CIP, Lima, Peru.



-Figure 2. Root-pulling device and a modified approach used for pulling potato plants.

Suggestions for data collection

Emergence, crop cover, tuber initiation

The effects of the drought treatment are dependent on the growth stage it coincides with. When the drought treatment should begin can be determined by crop emergence. Weekly counts of emerged plants can be gathered on a per-plot basis. Crop cover also can be measured using a standard grid[3] at weekly intervals. Sample data collecting sheets are given in Appendices 2 and 3 for potatoes and sweetpotatoes, respectively.

Tuber initiation of potatoes and root thickening (bulking) for sweetpotatoes can be determined scraping around the base of the stems of the border plants of the plot. For potatoes, these data can be collected from 3 weeks after planting at bi-weekly intervals. For

[3] For detailed instructions on how to gather crop-cover data, refer to CIP's Research Guide No. 30 and to Midmore, D.J. 1986. CIP Circular 14(1):79.

sweetpotatoes, it can be done from 5 weeks after planting, also at bi-weekly intervals.[4]

Root-pulling resistance

Root resistance to pulling can be recorded up to 45 days after planting for potatoes (i.e., 2 to 3 weeks after beginning a drought period imposed at maximum crop cover) and up to about 60 days after planting sweetpotatoes.

Pulling of individual plants can be done by wrapping a piece of cloth around the base of the plant and tying it to a rope, which is then attached to a simple scale or a dynamometer. This simplified approach is highly related to the use of a pulling device (Figure 2). As the plant is pulled out of the soil surface, the force required (resistance to pulling) is measured on the scale. It has been observed that for ease of manual pulling, sampling should be done when the individual plant resistance is less than 35 kg. Two workers are needed, one to pull and the other to record the data. Obviously, where growth analysis data on the pulled plants are to be recorded, more manpower is required. Recording time is about 2 to 3 minutes per plant. A minimum of 2-3 plants should be pulled separately in each plot. Sample data sheets are given in Appendices 2 and 3.

For the best results, no inter-row cultivation practices, which could disturb the root zones, should be done prior to pulling.

Yield

Per-plot yield can also be obtained on the same plots at maturity. The number of surviving plants, and a count of plants with tubers (or thick roots) can be done. Harvested tubers or roots can then be separated into size classes. Fresh weights per plot can be taken to calculate production per unit land area. If desired, sample dry weights can be obtained to calculate tuber or tuberous root dry-matter contents.

Other observations

Insect, fungus, and virus disease symptoms should be evaluated at least twice during the season; meteorological data obtained from the same site can help the researcher to interpret the data.

Selection

A preliminary selection and categorization of genotypes, is made according to of tuber-yielding ability and root-pulling resistance:

[4] A data-collecting format can be obtained from the data sheets given in CIP Technology Evaluation Series N° 1982-4.

resistant (higher yields and higher pulling resistance than the check); moderately resistant (higher yields or higher pulling resistance relative to the check); and, susceptible (lower yields or lower pulling resistance than the check).

Since drought-resistant traits are largely influenced by environment, genotypes should be tested in more than one season or location. The selected genotypes can be further tested on a large scale for their commercial acceptability and can be used in a breeding program.

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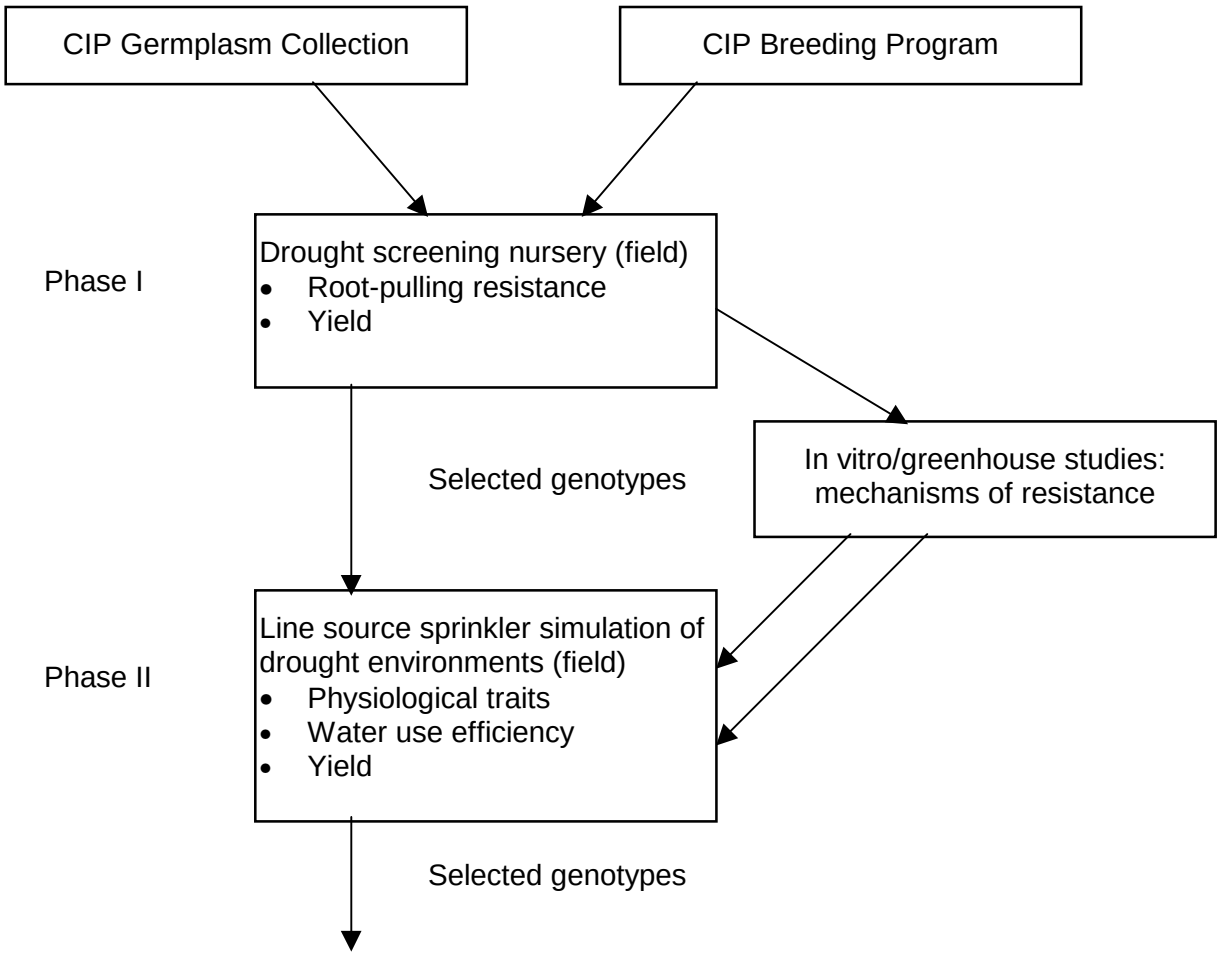
Appendices

Appendix 1. Flow chart of the procedures used in screening for drought at CIP.



Appendix 2. Potato: Growth analysis and pulling resistance

Program: ______________________ Location: ______________________ Planting date: ____________________ Plant age: ______________

Person responsible: ____________________ Season: ______________________ Sampling date: ____________________

			Foliage			Tubers								
				Sample		> 3.5 cm		< 3.5 cm		Sample		Roots		
Plot ID	Plant No.	Plant Ht.	Fresh weight	Fr. wt.	Dry wt.	No.	Wt.	No.	Wt.	Fr. wt.	Dry wt.	Fresh wt.	Dry st.	Pulling resistance Kg/plant

Appendix 3. Sweetpotato: Growth analysis and pulling resistance.

Program: ______________________________ Location: ____________________________ Planting date: _____________________ Plant age: ________________

Person responsible: ____________________ Season: ____________________________ Sampling date: ____________________

		Number or								Dry weight of						
Plant ID	Plant Ht.	stem	branch	inter.	Leaf	flower	fib. root	tub. root	Root length	aer. Fol.	Leaf	stem	flower	fib. root	tub. root	Pulling resist. (kg/plant)