Collecting vegetatively propagated crops (especially roots and tubers)

21

Z. Huaman[1], F. de la Puente[2] and C. Arbizu[2]
[1]*Department of Genetic Resources, CIP, PO Box 5969, Lima, Peru;*
[2]*CIP, PO Box 1558, Lima, Peru.*

Introduction

The most important root and tuber crops cultivated on a worldwide scale are potato (*Solanum tuberosum*), cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), yams (*Dioscorea* spp.) and taro (*Colocasia esculenta*), but there are many others that are of regional, national or local importance, in a total of over a dozen dicot and monocot families. Most of these originated in tropical or subtropical areas and are mainly used as sources of carbohydrates. Many minor root and tuber crops, such as turmeric (*Curcuma longa*) and arrowroot (*Maranta arundinacea, Tacca leontopedaloides*), are also used in folk medicine and as spices (Sastrapradja *et al.*, 1981). All these crops are vegetatively propagated. There are also vegetatively (or clonally) propagated crops that are not roots or tubers, e.g. bananas and sugarcane. A selection of the more important is given in Table 21.1.

Changes in the environment and in agricultural practices can cause particularly serious genetic losses in vegetatively propagated crops. This is because many set seeds only sparingly or not at all. Their capacity for adaptation is therefore lower than that of sexually propagated species, especially outcrossers, in which genetic recombination is much faster. Clonally propagated crops are also threatened by the accumulation of viruses in propagules (Lebot, 1992). It is therefore all the more important to conserve such species *ex situ* under controlled conditions. Among the Consultative Group on International Agricultural Research (CGIAR) commodity centres, the Centro Internacional de Agricultura Tropical (CIAT) maintains collections of cassava, the Centro Internacional de la Papa (CIP) potato and sweet potato, the International Institute of Tropical Agriculture (IITA) cassava and yams and the International Network for the Improvement of Banana and Plantain

Table 21.1. Selected vegetatively propagated herbaceous crops and the plant parts (•) suitable for germplasm collecting (Purseglove, 1968, 1972; Cardenas, 1969; Sastrapradja *et al.*, 1981; Tootill, 1984; Leon and Withers, 1986; National Research Council, 1989).

	Stools	Rhizomes	Bulbs	Offsets	Shoots	Suckers	Tubers	Stem cuttings	Bulbils	Stolons	Storage roots
Acorus calamus	•	•	–	–	–	–	–	–	–	–	–
Allium sativum	–	–	•	–	–	–	–	–	•	–	–
Ananas comosus	–	–	–	–	•	•	–	–	–	–	–
Agave spp.	–	–	–	–	–	•	–	–	•	–	–
Arracacia xanthorrhiza	–	–	–	•	•	–	–	–	–	–	–
Asparagus officinalis	–	•	–	–	–	–	–	–	–	–	–
Canna edulis	–	•	–	–	–	–	•	–	–	–	–
Colocasia esculenta	–	–	–	–	–	•	•	–	–	–	–
C. gigantea	–	–	–	–	–	•	–	–	–	–	–
Curcuma longa	–	•	–	–	–	–	–	–	–	–	–
Dioscorea spp.	–	–	–	–	–	–	•	–	–	–	–
D. bulbifera	–	–	–	–	–	–	–	–	•	–	–
D. esculenta	–	–	–	–	–	–	–	•	–	–	–
Eleocharis dulcis	–	–	–	–	–	–	–	–	–	•	–
Fragaria vesca	–	–	–	–	–	–	–	–	–	•	–
Hedychium coronarium	–	•	–	–	–	–	–	–	–	–	–
Ipomoea batatas	–	–	–	–	–	–	–	•	–	–	•
Lepidium meyenii	–	–	–	–	•	–	–	–	–	–	–
Manihot esculenta	–	–	–	–	–	–	–	•	–	–	–
Maranta arundinacea	–	–	–	–	–	•	–	–	–	–	–
Mirabilis expansa	–	–	–	•	–	–	–	•	–	–	•
Musa spp.	–	–	–	–	–	•	–	–	–	–	–
Oxalis tuberosa	–	–	–	–	–	–	•	•	–	–	–
Pachyrrhizus ahipa	–	–	–	–	–	–	•	–	–	–	–
Polymnia sonchifolia	–	–	–	•	–	–	–	•	–	–	–
Saccharum ssp.	–	–	–	–	–	–	–	•	–	–	–
Sansevieria ssp.	–	•	–	–	–	–	–	–	–	–	–
Solanum spp.	–	–	–	–	–	–	•	•	–	–	–
Tacca leonto-pedaloides	–	•	–	–	–	–	–	–	–	–	–
Tropaeolum tuberosum	–	–	–	–	–	–	•	–	–	–	–
Ullucus tuberosus	–	–	–	–	–	–	•	•	–	–	–
Xanthosoma nigrum	–	–	–	–	–	•	–	–	–	–	–
Vanilla planifolia	–	–	–	–	–	–	–	•	–	–	–
Zingiber officinale	–	•	–	–	–	–	–	–	–	–	–

(INIBAP) bananas and plantains. Collections of vegetatively propagated crops, whether in international centres or national programmes, are commonly stored in field gene banks or *in vitro*.

It is usually necessary to collect vegetative material of clonally propagated crops, normally the farmer's planting material. Even when possible, seed collecting is not necessarily desirable, as it may result in the loss of the specific gene combinations that make a clonally propagated variety distinctive. This presents several difficulties at different stages of collecting (Hawkes, 1975, 1980; Ford-Lloyd and Jackson, 1986), but all can be overcome by careful planning and appropriate techniques (Table 21.2).

Planning a collecting expedition

A particularly important aspect of the planning of expeditions to collect vegetatively propagated crops such as roots and tubers will be determining the most appropriate timing of the visit. Some of these crops, and especially their wild relatives, also produce seeds, though often only very few. An early decision that will therefore have to be made is thus whether the aim is to collect seed samples or vegetative material. The timing may be quite different. The decision will depend on:

- *The breeding system of the species.* Autogamous species can be collected as seeds without breaking up gene combinations.
- *Whether a population structure exists.* This will not be the case for traditional varieties clonally propagated for centuries but may still obtain for sexually fertile, outcrossing wild species.
- *The purpose of the collecting.* Each seed in a collection is potentially a new variety (farmers routinely use such open-pollinated seeds to increase the diversity of their fields), whereas each vegetative sample of a crop will represent an established variety. The former will be useful if the germplasm is to flow directly into a breeding programme, for example, the latter if the purpose of collecting is the documentation of present varietal diversity or the testing of adaptation of existing landraces.

In most cases, the vegetative parts that are collected for genetic resources conservation will be those that are used by farmers to propagate the crop. If this is also the economically useful product, the harvest season will be the appropriate time for collecting. Collecting storage organs which are immature or which are already sprouting may result in significant loss of viability during transport. Because a certain amount of precision is therefore needed and because the harvest season may vary considerably both between areas and between years, local information is vital at the planning stage in deciding on the best time to collect, as indeed on which areas to visit. Of course, at the best time for tuber collecting in such crops as potatoes (i.e. during dormancy),

Table 21.2. Solutions to problems at different stages of collecting vegetative material.

Stage	Problem	Solution
Searching	As underground structures, roots and tubers can be difficult to find and laborious to collect. They must be collected mature, which usually means when above-ground parts are dead	Use local information to time and direct the collecting. Time collecting when above-ground parts are just dying down. If resources allow, mark sites during a preliminary visit and revisit
Sampling	Genetic variation is difficult to estimate: above-ground parts will have died off and the material to be examined will have to be dug up. Without careful examination of the available variation, there may be excessive sampling of some clones, and some may be missed altogether	For wild species, spend sometime investigating the average extent of clones, and then avoid taking samples too close to each other at any given site. For crops, consult farmers for a thorough account of the variation available locally and collect in selective fashion in consultation with them
Transport	Planting material is often bulky and would thus be awkward to transport even if no great care were necessary to keep it viable, which is often not the case. The risk of moving pests along with the germplasm is greater than with seed	Obtain information on quarantine risks and ways to minimize them. Collect sound, mature material and take simple precautions for storage during transport, keeping transit time to a minimum. Alternatively, collect *in vitro* or transit material through an intermediate quarantine centre, taking *in vitro* samples there. Follow *FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm*

there will be no above-ground parts visible. This is perhaps not much of a problem for crops, but becomes serious in the case of wild relatives, demanding either a two-stage collecting effort or, as suggested by Hawkes (1975), a single visit when the plants are only just dying down.

There are many different types of plant material that can be used to propagate crops vegetatively. Definitions and examples may therefore be helpful, and some are given in Box 21.1 (Purseglove, 1972; Tootill, 1984; Bell, 1991).

Table 21.1 shows what parts of a selection of vegetatively propagated herbaceous crops may be collected for germplasm conservation. Woody species are considered separately in Chapter 23. In some cases, the collector may have alternatives and a choice will have to be made. One important distinction is between perennating organs like bulbs and tubers and growing parts like stem cuttings and suckers, which are generally more difficult to keep alive. Perhaps the main choice to be made in this context, however, is that between collecting the entire organ or removing meristematic tissue and maintaining this *in vitro*. Though this alleviates the problem of transporting large quantities of bulky material,

Box 21.1
Some definitions of vegetative propagating structures

- **Bulb** (e.g. *Allium*) – A short, fleshy, underground shoot, mostly made up of colourless, swollen leaf bases and scale leaves enclosing next year's bud. A **bulbel** or **bulbet** is a small bulb arising from a parent bulb.
- **Bulbil** – A small bulb arising in an axil on the aerial part of a plant.
- **Corm** (e.g. *Crocus, Gladiolus*) – A short, swollen, annual underground stem of several nodes and internodes, with that of the next year forming above it at the base of each flowering stem. A **cormel** is a corm arising vegetatively from a parent corm.
- **Knob** (e.g. *Amorphophallus campanulatus*) – Sprout from the main tuber.
- **Offset** (e.g. *Sempervivum tectorum*) – Small plantlet taken from the base of the above-ground part of the main stem.
- **Rhizome** (e.g. *Acorus, Zingiber*) – An underground stem, usually horizontal and often elongated, that is distinguished from a root by the presence of nodes and internodes and/or the presence of buds in the axils of reduced, scale-like leaves.
- **Shoot** (e.g. *Ananas*) – The apical or lateral meristematic dome together with the leaf primordia, emerging leaves and subadjacent stem tissue.
- **Stem cutting** (e.g. *Ipomoea batatas*) – Section of a mature stem with several leaves and their axillary buds.
- **Stolon** or **runner** (e.g. *Fragaria vesca*) – A trailing stem, capable of forming roots, shoots or both (and hence a new plant) from its axillary buds.
- **Stool** (e.g. *Acorus*) – A clump of roots or rootstocks (short, erect underground stems, like vertical rhizomes).
- **Storage root** or **root tuber** (e.g. *Mirabilis expansa*) – A fleshy, secondarily thickened root, without buds (except sometimes adventitious buds giving rise to suckers).
- **Sucker** (e.g. *Agave*) – A shoot arising adventitiously from a root, often at some distance from the main plant.
- **Tuber** (e.g. *Colocasia, Solanum*) – A swollen, subterranean stem, with numerous buds or 'eyes'.

and coupled with disease indexing may also overcome quarantine risks, it poses technical and logistical questions that need to be addressed at an early stage (Chapter 24).

Sampling procedures

Wild populations of root and tuber crop relatives are likely to be different from those of autogamous species not producing such structures only in so far as single clones may be quite extensive. Sampling vegetative material of the former will therefore not be any different from sampling seeds of the latter, except that care will have to be taken not to sample large clones excessively. It will also not be possible to collect as many propagules per plant. Hawkes (1980) suggests collecting as a bulk sample two to four vegetative propagules from each of 10–15 randomly chosen

individual clones (more if time allows, up to 25; fewer if the propagules are very large). Individual clones may be identified by their appearance or duplication at any given site minimized by taking samples not too close together, based on an initial assessment of the likely average extent of clones. The latter approach may be taken to an extreme by collecting from only one or two plants per site but visiting many more sites. A bulk seed sample should also be taken, when possible, and given a separate collecting number.

In contrast to the situation in the wild, a field of a root or tuber crop will probably not display a population structure at all. There will be a mixture of many different genotypes in a traditional farmer's field (e.g. Jackson *et al.*, 1980), each being the result of intensive selection by farmers over many generations. Random sampling of such a field, the usual method for sexually reproducing species, will not be appropriate, as it will over-represent abundant clones at the expense of rare ones. The recommended procedure is selective sampling, i.e. to identify all 'morphotypes' in a field or market and collect two to four propagules of each one, repeating the process at each sampling site. Duplicates between sites can be identified later, when the material is grown out. Ford-Lloyd and Jackson (1986) define the morphotype as being composed of plants which are apparently phenotypically identical, without implying genetic identity. If it is possible to collect seed, numbering of samples should be as follows (Hawkes, 1980): give the same collecting number if seeds are taken from the same plant(s) from which a vegetative sample has been taken; give a separate collecting number if the seeds are taken from more than one randomly chosen morphotype and bulked, the recommended sampling procedure for outcrossers.

In crops like potatoes, oca, ulloco and mashua, phenotypic variation in the tubers is high, which means that selecting potentially different cultivars growing in the same field is relatively easy. However, in many root and tuber crops, visible phenotypic differences among cultivars are few but one can find significant genetic variation when other, more cryptic, characters are considered. Thus, for example, morphologically similar cultivars may have different isozyme alleles at some loci, or display different reactions to biotic or abiotic stresses. In these cases, more samples need to be obtained, combining selective and random sampling techniques. Working in Irian Jaya, Prain *et al.* (Chapter 38, this volume) relied on the farmer to identify sweet potato duplicates within a plot and also avoided similar material with the same name in nearby plots; however, in more distant plots, and if there were any doubts, the material was collected.

Local knowledge is crucial to the sampling process, just as it is crucial in deciding when and where to sample in the first place. Farmers will be aware of the extent of variation in their field, village and district, i.e. the number of distinct cultivars available in a given area, their names, appearance and properties. Hawkes (1975, 1991) suggests that market areas should be the units within which sampling of morphotypes should

be organized. Markets in towns serving a whole district will certainly be important sources of information on the varieties grown in the area, though not all varieties grown will be found on market stalls, some being kept solely for home consumption (Hawkes, 1991). On the basis of this kind of information, it may be feasible to attempt to collect all alleles occurring in the area, an ideal that is impossible to achieve in sexually reproducing crops. Chapter 38 describes how local knowledge has been brought to play during collecting of sweet potato in Irian Jaya.

Handling vegetative material in the field

Vegetative material can deteriorate rapidly after harvest. It is also easily damaged in transport. It must therefore be properly prepared and housed in suitable containers. Samples should also be free of insect damage and disease symptoms. If they consist of underground organs, they should be free of soil. Leon and Withers (1986) provide guidelines for the field handling of collected material of several vegetatively propagated crops (*Colocasia, Dioscorea, Hevea, Ipomoea, Manihot, Musa, Saccharum, Vanilla* and *Zingiber*).

Storage organs should be collected fully grown – dormant in crops like potatoes and yams. They should also be sound. Care should be taken to avoid mechanical damage when digging up underground parts with metal implements. Bruises can be cut out and treated with a mild alkali such as chalk, hydrated lime or wood ash, but in general damaged material should be avoided. If the tuber is too large, it may be possible to collect only a portion. In yams, this would be the head or proximal end, in taro and other aroids the crown of the tuber. Gentle drying in the sun can be followed by cleaning off excess soil and a fungicidal dusting or dip. Hawkes (1975) then advises wrapping tubers in a semipermeable material and keeping them under cool conditions, for example in insulated cool boxes. Expanded polystyrene containers covered with reflective mirror foil are suitable.

Storage organs that do not sprout soon after harvest generally require only to be placed individually in strong paper bags or newspaper and loosely accommodated in boxes with soft packing material. Material of some species is fragile and cannot survive if too many layers of samples are placed on top. In these cases, specially made crates are needed allowing the storage of single layers of samples in trays or shelves. There should be adequate ventilation and extremes of temperature should be avoided.

Growing tissues generally require more care. In general, stem cuttings should be taken from the middle portion of the stem and consist of several nodes. Leaf surface should be reduced to a minimum, but leaves should be snapped off at the junction with the stem rather than cut. Cut ends can be dipped in disinfectant to prevent rotting. Shoots, suckers and cuttings from fleshy stems keep reasonably well when the proximal end is wrapped in slightly wet paper towels or newspaper. They can then be

stored in insulated cool boxes. Samples should be examined regularly to keep the wrapping paper moist and excise developing roots. Sweet potato stem cuttings are usually about 30 cm long and contain at least six nodes. The growing tip and large leaves are discarded. Cassava stem cuttings (called 'stakes', and usually 40–50 cm in length) are not so sappy and tender as those of species like sweet potato and can be simply wrapped in paper bags. In sugarcane, which is not a root or tuber crop but is clonally propagated from stem cuttings much like cassava, cut ends are sealed by dipping in hot paraffin after a short hot-water treatment (50°C for 30 minutes) (Coleman, 1986). Collecting vegetative material of *Musa* is described in detail in Chapter 33.

Fern perennating structures may be collected as live material following the same basic precautions as for those of flowering plants. However, aquatic ferns like *Azolla* spp., which are important nitrogen sources, may be collected as fronds. Watanabe *et al.* (1992) describe methods for collecting and transporting *Azolla*. They suggest gathering the fronds a handful at a time, squeezing out excess water and draining on toilet paper. The material may then be placed in Petri dishes or plastic bags, which should be sealed and maintained in a cool place until arrival at the laboratory. Under such conditions, samples may survive up to two weeks. *Azolla* material is conserved in the vegetative state in liquid medium. Watanabe *et al.* (1992) also give details of different ways of maintaining collections.

Chapter 24 describes the concept of *in vitro* collecting, gives general guidelines and provides some examples. Other examples involving root and tuber crops may be found in Chapter 32. Two further examples are worth mentioning here. The *in vitro* collecting method developed at CIAT for cassava (R. Chavez, pers. comm.) consists in taking actively growing vegetative buds or terminal stem cuttings from branches without flowering buds. Explants of 1.0–1.5 cm are immersed in 70% ethanol for 5–15 minutes and then surface-sterilized by immersion in a 0.5% solution of calcium hypochlorite for 5 minutes. Finally, they are rinsed with cool boiled water. Explants are inoculated into semisolid culture medium (MS or 4E) containing an antibiotic such as rifampicin in a small wick of filter paper. In contrast, the *in vitro* methods tested at CIP for collecting sweet potatoes have so far not produced high rates of survival of the cultures. A simple method that has been partially successful consists of taking cuttings containing one node with axillary buds; they are surface-sterilized and introduced into a test tube containing 1 ml of antibiotic solution (100 ml distilled water + 0.025 g streptomycin). Particularly high losses due to contamination have been noticed in sweet potatoes with thin or very pubescent stems.

After the mission

If there are transport and other delays, or if storage organs develop rots, it may be worthwhile planting out the material at some kind of way station in the field. Cuttings would be planted out as a holding operation, storage organs for conversion to stem cuttings.

FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm are available for several clonally propagated crops (Chapter 16). In general, they recommend shipment of collected material as sterile, pathogen-tested *in vitro* plantlets, if necessary via intermediate quarantine stations. Thus, cassava might be collected as stakes and transported in this way to an intermediate centre where the material would be planted out and meristem tips transferred to *in vitro* culture for eventual removal to the germplasm centre for indexing and storage in field and/or *in vitro* gene banks. Chapter 33 describes a similar procedure for *Musa*. If the material is not moved in this way, all samples collected should be inspected to eliminate organs or organ parts that are rotten or damaged, and further regular inspections will be necessary, with all infected material incinerated, as long as it is grown under quarantine conditions.

On arrival at its final destination samples of growing material will need to be planted out fairly quickly. Dormant tubers will need to be stored under appropriate conditions. *In vitro* plantlets will need to undergo propagation. All this requires that precise and timely preparations be made for receiving the material. This should be done at the planning stage if potentially damaging delays in dealing with the material are to be avoided.

References

Bell, A.D. (1991) *Plant Form.* Oxford University Press, Oxford.

Cardenas, M. (1969) *Manual de plantas economicas de Bolivia.* Imprenta ICTHUS, Cochabamba.

Coleman, R.E. (1986) Sugarcane. In: Leon, J. and L.A. Withers (eds) *Guidelines for Seed Exchange and Plant Introduction in Tropical Crops.* FAO Plant Production and Protection Paper 76. pp. 169–171. FAO, Rome.

Ford-Lloyd, B. and Jackson, M. (1986) *Plant Genetic Resources: An Introduction to Their Conservation and Use.* Edward Arnold, London.

Hawkes, J.G. (1975) Vegetatively propagated crops. In: Frankel, O.H. and J.G. Hawkes (eds) *Crop Genetic Resources for Today and Tomorrow.* pp. 117–121. Cambridge University Press, Cambridge.

Hawkes, J.G. (1980) *Crop Genetic Resources Field Collection Manual.* IBPGR and EUCARPIA, Rome.

Hawkes, J.G. (1991) Theory and practice of collecting germplasm in a centre of diversity. In: Engels, J.M.M., J.G. Hawkes and M. Worede. (eds) *Plant Genetic Resources of Ethiopia.* pp. 189–201. Cambridge University Press, Cambridge.

Jackson, M.T., J.G. Hawkes and P.R. Rowe (1980) An ethnobotanical field study of primitive potato varieties in Peru. *Euphytica* 29:107–113.

Lebot, V. (1992) Genetic vulnerability of Oceania's traditional crops. *Experimental Agriculture* 28:309–323.

Leon, J. and L.A. Withers (eds) (1986) *Guidelines for Seed Exchange and Plant Introduction in Tropical Crops.* FAO Plant Production and Protection Paper 76. FAO, Rome.

National Research Council (1989) *Lost Crops of the Incas.* National Academy Press, Washington DC.

Purseglove, J.W. (1968) *Tropical Crops. Dicotyledons.* Longman, London.

Purseglove, J.W. (1972) *Tropical Crops. Monocotyledons.* Longman, London.

Sastrapradja, S., N. Wilijarmi-Soetjipjo, S. Donimihardja and R. Soejono (1981) *Root and Tuber Crops.* IPBGR, Rome.

Tootill, E. (ed.) (1984) *Dictionary of Botany.* Penguin, London.

Watanabe, I., P.A. Roger, J.K. Ladha and C. van Hove (1992) *Biofertilizer Germplasm Collections at IRRI.* IRRI, Manila.