Collecting germplasm from trees — some guidelines

The process of domestication begins with the farmers. The farmers, the ultimate users and beneficiaries of improved agroforestry trees, are therefore the only ones who can tell researchers which trees they value the most, why these are their 'priority species', what tree products benefit them most and how they would like to see the trees 'improved'. It is only after these priority species have been identified that germplasm collections can have the impact they should. And in this article, ***Ian Dawson*** *and* ***James Were*** *offer readers some step-by-step guidelines on how to go about making collections.*

There are many reasons for collecting tree germplasm. There may be a need for planting material that will be distributed immediately to users—farmers or horticulturists or extension workers. With the rapid rate of deforestation throughout the tropics, many species of trees—and their genetic wealth—are endangered and the collection may be to conserve their germplasm for posterity. In addition, germplasm may be collected because it is needed for tree-improvement programmes.

Germplasm collection is a key step in any process to domesticate trees; the genetic variation of a species should be present in the collected germplasm, and this is needed for the selection and improvement that are part of domestication. Many tropical trees have undergone little or no domestication, so the best source of germplasm for improvement of these species is wild populations. However, collecting is also often done in exotic stands, because they are relatively easy to get at and they provide a good comparative base in trials to assess the performance of different sources of germplasm.

There are as many strategies for collecting germplasm as there are reasons for doing it. Finding the right strategy will depend on the purpose of collection, the biology of the species and the ability to select for desirable characteristics during sampling. Normally, the aim of collection is to sample germplasm that is as genetically representative as possible of a population (or provenance). This is called

Ian Dawson is a germplasm specialist with ICRAF, PO Box 30677, Nairobi, Kenya; email: i.dawson@cgnet.com; fax: +254 2 521001. James Were is a seed physiologist at ICRAF in Kenya.

systematic sampling, and it means collecting seed from many trees in an individual population. This is the strategy that national partners and ICRAF used to collect germplasm of *Calycophyllum spruceanum* and *Guazuma crinita* in Peru and *Prunus africana* in Cameroon (see Ian Dawson's article p 15–17). In addition to systematic sampling within populations, the researchers also collected from several populations for each species, the idea being to get germplasm that represented the geographical ranges of the 3 tree species.

Sometimes in the course of the collection, if researchers decide that important characteristics have high heritability and can therefore be selected for at the time of sampling, they can do phenotypic selection of trees within populations. This increases the chances of capturing superior material for improvement programmes; this method is called 'targeted collection'. This is the approach researchers used for their collections of germplasm of *Sclerocarya birrea* and *Uapaca kirkiana* in southern Africa (see news item p 28). For both species, researchers collected seed from trees that villagers identified as producing fruit with superior characteristics. In addition, the collection team systematically sampled seed from a number of populations so that they could assess, in subsequent field trials, whether this kind of phenotypic selection during collection was efficient.

Occasionally, collectors may also do vegetative sampling to collect superior phenotypes, or because trees do not bear seed at the time of sampling. This approach may be useful for the targeted collection of fruit trees such as *Sclerocarya birrea* or *Uapaca kirkiana,* or for endangered species such as *Prunus africana,* for which mature, seed-bearing trees can be difficult to find in some locations.

Although germplasm is sometimes collected in these targeted or vegetative ways, we recommend these in only very specific cases. This is

A glossary for germplasm collection

exotic: a tree growing outside its native range, normally as a result of planting by people. Such populations often have a narrow **genetic base**.

germplasm: genetic material, which can be seed, pollen, vegetative propagules or other material, though normally seed.

genetic base: the amount of genetic variation in a species or population. During collection, maximizing the genetic base sampled can prevent **inbreeding depression** in future generations.

heritability: the proportion of the total variance of a characteristic that may be accounted for by genetic factors. If a character is not highly heritable, most variation can be accorded to environmental factors. **Phenotypic selection** cannot be usefully practised on such characters during collection.

inbreeding depression: a decline in the vigour of a species as a result of decreased levels of heterozygosity at individual loci. This may occur in species that are preferentially **outcrossing** when their **genetic base** is reduced. This negative effect may be prevented by maintaining the widest possible genetic base during the collection of populations.

microsymbiont: bacteria or fungi associated with a tree, existing in a relationship that brings benefits to both. For many trees, such organisms are essential for good growth.

orthodox seed: seed that can remain viable for long periods if processed and stored in the appropriate manner (normally seed should have a low moisture content and be kept at low temperatures).

outcrossing: the production of offspring by the transfer of pollen between individuals rather than by self-pollination. Outcrossing often results in high levels of heterozygosity in populations. Many trees are preferentially outcrossing.

phenotypic selection: the choice of individuals based on their physical appearance, which may or may not reflect their genetic makeup, depending upon the **heritability** of characters; sometimes referred to as targeted collections.

population: normally a group of individuals growing in the same general location, which are potentially interbreeding.

provenance: a term generally used interchangeably with the term **population,** although it relates to geographic rather than genetic confines.

recalcitrant seed: seed that loses its viability if stored for any length of time, even under conditions that are normally conducive to seed longevity.

seed viability: the proportion of seed that can produce viable plants. Often this is estimated by testing levels of seed germination.

systematic sampling: collecting seed from many randomly selected but well-spaced trees in an individual population. Such collection provides representative sampling of a population and the widest possible **genetic base**.

vegetative sampling: the collection of vegetative material, such as stems or root cuttings, from a species.

The logistics of germplasm collection in remote areas can be daunting—and often perilous—but following some basic rules of thumb can still help ensure success.

because these approaches have a potential disadvantage—they can lead to a narrowing of the genetic base of collected populations. Generally, therefore, the best approach will be systematic sampling of seed. Below are a few standard guidelines for the systematic sampling of seed from an individual population. These guidelines are summarized into appropriate steps before, during and after collection. Although certain steps alter for targeted seed sampling or vegetative collection, many considerations remain the same. For more details on collecting germplasm from trees, see FAO, Forestry Resources Division guidelines (1995).

Before collection

1. Decide on the purpose for which germplasm is required.
2. Find out if suitable and well-documented germplasm is already available from other sources. Inform others of your plans, to avoid duplication of efforts in collecting from the same area.
3. *Develop a collection strategy*—determine the where, when and how of collection.

- *Where*—find out the geographical and ecological areas where a species grows and from what areas it can be collected (literature, herbaria, field exploration).
- *When*—decide on the best time for collection. This may require a prior visit to a site to identify the period when seed is mature. Herbarium specimens often give dates of fruiting or collection. Trees may seed only in certain years and the timing of seed production may also vary between years and regions. For species with a prolonged fruiting season, more than 1 sampling time might be needed to avoid collecting only the early fruiting trees.
- *How*—estimate the quantity of seed required from the collection, to help determine the appropriate sampling strategy. In addition, decide if seed from individual trees should be kept separate during collection or bulked to form a single population sample. For research trials, individual tree collections are sometimes needed, while in other cases a bulk collection from a population suffices. If material is being collected for immediate distribution to farmers or other users, a bulked collection strategy may make handling easier.

4. Find out the requirements for handling seed of a species being collected, including necessary seed treatments to ensure maximum seed viability. If seed is orthodox and kept under the appropriate storage conditions after collection, it may remain viable for many years before being planted. If seed is recalcitrant, however, it is necessary to prepare for immediate planting after collection.
5. Ensure that all necessary equipment for collection is available. Collection from trees may require specialist tools, such as pruning saws and tree-climbing equipment. Use open-weave collection bags rather than plastic ones, to allow aeration.
6. Ensure that the necessary permission for collection is obtained. If collections are to be from communal or private land, it is necessary to obtain the permission of the head of the community or the land-owner. For large-scale collections, permission must also be sought from the relevant government bodies. If collections cross national boundaries, permission for the export of germplasm between countries may be required.

During collection

7. *Develop a sampling strategy*—to ensure representative sampling of a population, the following guidelines should be adopted:

- *Number of trees sampled and their selection*—collect seed from at least 30 trees. If possible, collect from more individuals. No selection criteria should be used, although normally only those trees producing reasonable quantities of seed are chosen.
- *Collection from crown*—for each tree, sample different points in the crown, especially if the species is insect pollinated. This is because individual pollinators carrying pollen from different potential fathers may visit only part of a tree crown. So seed from different points in the crown may be different genetically. If it is not possible

to sample directly from the tree canopy, seed or fruit that has fallen naturally and is lying underneath trees may be collected.

- *Spacing*—ensure a reasonable spacing between sampled trees to reduce the likelihood of collecting closely related individuals. Ideally, individual trees should be spaced by a distance greater than that associated with normal seed dispersal—usually at least 50 m apart.
- *Bulking of seed*—if seed from individual trees is bulked during collection, each tree should contribute roughly the same amount of seed. Bulking can always be done later so it may be worth keeping seed from individual trees separate.
- *A pragmatic approach*—although the above represent ideal sampling criteria for a population, be pragmatic and realistic when in the field.

8. Ensure that, wherever possible, physiologically mature seed is collected. Otherwise, the viability of seed may be very low.

9. Do not collect so excessively from a population that its survival through natural regeneration is threatened.

10. For tree species with associated microsymbionts, take soil or root samples—or both—during seed collection. This is particularly important for nitrogen-fixing legumes with root nodules containing *Rhizobium* or *Bradyrhizobium*.

11. *Document the work*—ensure that accurate and adequate records are kept during collection. A collection form designed prior to collection is useful. Documentation is essential, for example, in relocating populations that trials show to have useful characteristics and for identifying gaps in conservation collections. *As a minimum*, the following should be recorded for a population:

- species name
- collection date
- individuals carrying out the collection
- population location, including name of location and directions to reach the site (where possible, accurate latitude and longitude measurements should be recorded from maps or by using a Global Positioning System receiver)
- number of trees collected from at each site
- the approximate average distance between trees
- a unique identifier for each collected sample (normally a number, which should be used to label seed during and after collection)

Other data that should be record for research and conservation collections include

- altitude, soil type and depth of water table for dry areas (the latter can be determined from a well near the collection site)
- morphological characteristics of trees in the population
- density of trees in the collected stand
- status of the population (natural, naturalized or planted)
- abundance of the species in the area
- type of vegetation (primary or secondary)
- associated species
- human disturbance (if any)
- local guides
- local name of the species
- local uses
- maturity of collected seed
- presence of any pests or diseases

After collection

12. Back at base, ensure seed is correctly processed and placed under storage conditions that maintain optimum viability, until required for planting. Normally, the viability of seed is tested before it is placed in storage.

13. Keep a record of the collection exercise. Distribute this report to individuals involved in collection and others who may require a record of, or be interested in, the collection exercise. The collection records should include

- objectives of the collection
- the approach used
- all documentation taken
- recommendations for follow-up work

14. For large-scale collections, duplicate germplasm at additional storage sites for safety purposes.

The above strategy will provide collections of germplasm that should be representative of a population and have the widest possible genetic base. The former is important because it means that future collections of the same population, using a similar collection strategy, should result in seed that is similar genetically in its characteristics. A wide genetic base is particularly important for trees because the majority of species are preferentially outcrossing. A wide base can therefore prevent inbreeding depression in future generations as germplasm is distributed to users. In addition, a wide genetic base for agroforestry trees provides an adaptive capacity to changing user requirements and varying environmental conditions (Simons and others 1994).

References

FAO, Forestry Resources Division, Forestry Department. 1995. Collecting woody perennials. *In:* Guarino L, Ramanatha Rao V and Reid R, eds. *Collecting plant genetic diversity: technical guidelines.* Wallingford, UK: CAB International. p 485–509.

Simons AJ, MacQueen DJ and Stewart JL. 1994. Strategic concepts in the domestication of non-industrial trees. *In:* Leakey RRB and Newton AC, eds. *Tropical trees: the potential for domestication and the rebuilding of forest resources.* London, UK: HMSO. p 91–102.