Collecting *in vitro* for genetic resources conservation

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The need for new collecting techniques

Experienced germplasm collectors will base their plans on the best information that is available on the constraints that can limit the effectiveness of the proposed project. There may only be a limited 'window of opportunity' within which the mission can be carried out, for example due to the weather or the limited availability of personnel. When the target germplasm is threatened by changing land use or other factors affecting its future availability, the uniqueness of the opportunity is further emphasized. Despite experience and good planning, however, a collecting project can still meet difficulties which have to do with biological features of the plant parts that would normally be collected. There may simply be insufficient material because of a poor growing season, general scarcity of the plant in the target region, grazing of the plant by animals (likely in the case of forage plants), damage by pests or diseases or immaturity of the material.

These problems can apply to both seeds and vegetative propagules that have a seasonal pattern of development. Plant parts that are not strictly organs of propagation or perennation, for example budwood, are more flexible in that some collectable material is likely to be available at all times. However, being growing tissues, they are less likely to be able to survive a long journey back to the gene bank. This problem of deterioration by natural processes and attack by pests is shared by recalcitrant seeds, which may germinate or deteriorate to a non-viable condition in transit. A final problem, shared to some degree by all types of plant material but most serious in the case of large vegetative propagules and recalcitrant seeds and their fruits, is excessive weight and bulk. The cost and inconvenience of transporting large amounts of material can be a severe limitation on the scope of a collecting mission.

In the face of these potential problems, collectors need as many options as possible. It is in this context that the possibility of using an *in vitro* collecting method began to be considered. In 1982, the International Board for Plant Genetic Resources (IBPGR) constituted an Advisory Committee on *In Vitro* Storage to examine the status of *in vitro* conservation technology and to highlight opportunities for developing and applying new approaches to solve bottleneck problems in the conservation of plant genetic resources. The difficulties associated with collecting germplasm of the two major categories of problem species – i.e. those with recalcitrant seeds and those that are vegetatively propagated – were recognized as such bottlenecks. It was also noted, however, that all types of plant could benefit from improved collecting techniques.

In order to examine this topic in detail, a subcommittee of the *in vitro* advisory committee met in 1983. This included experts in germplasm exploration, in the genetic resources of problem crops and in the application of *in vitro* technology to plant propagation and conservation. It was evident that *in vitro* techniques could assist in the safe conservation of problem crops (Withers, 1980, 1982). The basic premise that the subcommittee examined was that some aspects of *in vitro* technology, namely inoculation and plant regeneration, could also be adapted to allow problem material to be collected. This would, however, be a 'holding operation', rather than a full-scale *in vitro* propagation exercise.

With the help of some preliminary experiments carried out in the institute that hosted the meeting (School of Agriculture, University of Nottingham, UK), the subcommittee came to the conclusion that *in vitro* collecting had great potential. A report and recommendations were produced (IBPGR, 1984). The further development of collecting technologies for two model systems (*Theobroma cacao* budwood and *Cocos nucifera* embryos) ensued in research projects over the following years. These are described below. First, it is appropriate to analyse the principle of using *in vitro* inoculation and plant regeneration.

Adapting basic *in vitro* procedures

Useful reviews of *in vitro* culture methods include Sharp *et al.* (1983, 1984), Beversdorf (1990), Pollard and Walker (1990), Lindsey (1991) and Bonga and von Anderkas (1992). *In vitro* culture inoculation as carried out in the laboratory involves the following steps:

- selecting an appropriate inoculum tissue;
- cutting it to a suitable size;
- removing superficial soil and visible pests by washing;
- sterilizing the surface;
- washing to remove the surface sterilant;
- trimming away non-essential tissue and tissue damaged by sterilization;

- inoculating into the culture vessel containing nutrient medium and closure of the vessel;
- transferring to incubation conditions.

Sterilization and inoculation are carried out in an environment that protects the tissue from reintroduction of contaminants, usually a laminar air-flow cabinet. Incubation is carried out in a controlled environment in which temperature, light quality, light intensity and day length are regulated to allow optimum growth and development. The culture medium contains nutrients to support growth and development.

Development of the inoculated tissue will depend largely on the composition of the culture medium. Under some conditions the inoculum will be directed towards multiplication, and under others towards regeneration of an independent plant. Thus, a shoot explant could be induced either to produce multiple lateral shoots, which could be separated and multiplied further, or to produce roots, allowing its transfer to soil.

This pattern applies to 'non-adventitiously' regenerating systems, i.e. those capable of producing a plant from pre-existing shoot primordia, such as in a stem nodal explant or a zygotic embryo. Alternatively, it is possible to induce buds *de novo* (i.e. 'adventitiously') from virtually any tissue, opening up the possibility of rapid mass clonal propagation. Generally, genetic conservation applications of *in vitro* technology favour the non-adventitious propagation system. This choice is made on the assumption that genetic instability as a result of somaclonal variation is likely to be lower in such systems (Scowcroft, 1984). Although non-adventitious systems are more suitable, tissue that is likely to regenerate adventitiously should not be rejected if it is the only material available. This is an opportunity for imaginative use of the great flexibility of *in vitro* culture. For example, a piece of leaf tissue could produce plants via somatic embryogenesis or an unfertilized ovary could be cultured and pollinated *in vitro* (Dunwell, 1985; Tisserat, 1985).

In examining the stages of *in vitro* inoculation and plant regeneration from the point of view of germplasm collecting, the first point to remember is that *in vitro* collecting is a holding operation rather than propagation *per se*. It should be aimed at maintaining the material as well as possible under field conditions for a relatively limited period. A second point is that working in the field imposes limitations on what is feasible. Therefore, a decision should be made at the outset about which stages it is absolutely necessary to carry out in the field. Other operations can be done at an earlier or later time, at locations where more appropriate conditions can be provided. It may be necessary to introduce alternative or additional steps to overcome particular limitations imposed by working in the field. To illustrate how this kind of analysis can be carried out, each of the steps in the inoculation procedure listed above will be examined in turn.

1. *Selecting an appropriate inoculum tissue.* If possible, a tissue robust enough to withstand sterilization should be selected. The main choices

are: herbaceous shoots, woody shoots, zygotic embryos/embryo axes and seeds.

2. *Cutting it to a suitable size.* Surface injury should be minimized but the opportunity should be taken to remove very dirty, infested or damaged outer tissues.

3. *Removing superficial soil and visible pests.* Copious supplies of water for this purpose may not be available in the field but the water does not need to be very pure or sterile.

4. *Surface sterilization.* Sterilants should be safe and easy to transport and of low toxicity to the plant tissue in situations where adequate sterile water for subsequent washing is not available (mercury salts and strong oxidizing agents may be unsuitable in this respect). Unconventional sterilants, such as drinking-water purifying tablets or agricultural fungicides, and combinations of more than one sterilant, each at low concentration, could be used. Sterilization and subsequent stages will probably have to be carried out without benefit of an aseptic environment (but see below). This has implications for the container used for sterilization, one with a securely fitting lid being more suitable than, say, an open beaker. Sterilization is a step which can be repeated after the collected tissue reaches the laboratory. Therefore, it is worth considering simple, short-term methods to maintain adequate cleanliness rather than risking harsher treatments that might damage the tissue. Another consideration here is that many meristematic plant tissues are free of microbial contamination, being protected by overlying leaves, bracts, seed coat, etc. Advantage can be taken of this by selecting an explant that can be surface-sterilized to remove gross contamination and then dissected to remove outer tissues. The introduction of contamination into inner tissues can be avoided by frequent changes of dissecting instruments and by working carefully and swiftly.

5. *Washing to remove the surface sterilant.* The amount of washing necessary will depend on the strength and toxicity of the sterilant used. If post-sterilization washing is included in the procedure, adequate sterile water will be needed and the technique used must avoid reintroducing microbial contaminants. Also, any residual effects of sterilants (e.g. continuing fungicidal effect) will be lost by washing.

6. *Trimming away non-essential tissue and tissue damaged by sterilization.* This is another step which might reintroduce contaminants and should be avoided if possible.

7. *Inoculation into the culture vessel containing nutrient medium.* Several factors must be considered here, including the type of vessel, the number of pieces of tissue inoculated into each vessel and the type of culture medium used. The vessel must be portable and therefore robust but not excessively heavy. Plastic materials are more suitable than glass and, in certain circumstances, something as simple as a plastic bag may suffice. Placing several inocula in a single vessel will be more efficient of space but will also increase the risk of cross-contamination. The efficiency of sterilization, the length of time the tissue is in transit, the

susceptibility of the tissue to destructive injury by contaminants and the possibility of effective resterilization will all influence this decision. The culture medium must be designed to suit its purpose. If it is intended to promote development (e.g. germination of an embryo or outgrowth of axillary buds), suitable growth regulators should be included along with other nutrients. This might be the case, for example, in a collecting mission of relatively short duration or when the collecting environment and the nature of the explant allow inoculation with a low probability of contamination. If it is more logical to try to suspend development, a minimal medium or even one containing growth retardants would be appropriate for the collecting period, to be replaced by a standard medium once the material reaches the laboratory. A minimal medium, particularly one low in sucrose, is less likely to support the growth of residual microbial contaminants than is a complete medium. Antimicrobial additives may also be included in the culture medium to retard the growth and destructive effects of bacteria and fungi, but possible side-effects on the inoculum should be borne in mind. A choice will have to be made between liquid or solid medium. A liquid medium is more accessible to the tissue but is less effective in retarding growth of microbial contaminants. Also, vessels containing liquid medium must be closed more securely.

8. *Transfer to incubation conditions.* This stage will be far more lengthy and hazardous than the simple room-to-room transfer carried out in the laboratory. Every effort must be made to protect the inoculated material in transit. This will influence the choice of culture vessels used and of the container used to transport them. Attention should be given to the conditions likely to be experienced in transit (e.g. fluctuating temperatures, physical disturbance). There is the possibility of using refrigeration to retard deterioration in collected material that is adequately chilling-tolerant.

The stages of the *in vitro* collecting procedure have been analysed above from the point of view of the demands of inoculation under field conditions. A second set of considerations will relate to the constraints imposed by the nature, scale and duration of the collecting mission, the extent to which *in vitro* collecting is a central activity and the expertise of personnel. There are many variables to examine here and only illustrative examples will be offered. For a multi-species collecting mission, it will be necessary either to design different facilities and procedures to match the different needs of each of the materials to be collected, or to use a very general collecting approach to be followed up by different treatments in the laboratory. If *in vitro* collecting is a backup rather than the main approach used, the levels of replication and the extent of the resources and time given to it can be reduced accordingly. If the collecting mission personnel have only basic *in vitro* experience, the field operations should be designed to match their level of expertise, leaving as much as possible to be carried out later in the laboratory by

experienced people. This is, in fact the most likely situation, since it is more logical and feasible to train collecting experts in the principles of *in vitro* inoculation than to train *in vitro* technologists to enable them to carry out specialized collecting.

As well as adapting the inoculation procedure, it is necessary to adapt the equipment used, taking into account essential requirements and the constraints imposed by having to carry all items to the field, where services will be limited. What is certain is that the equipment used should be robust, simple to operate, as maintenance-free as it can be made and, if possible, multipurpose. Thus, a packing-case to hold instruments and culture vessels can also serve as a work bench and/or inoculation hood. The examples of the successful application of *in vitro* collecting given below illustrate the great flexibility of *in vitro* collecting in terms of the equipment that can be used and the degree of sophistication of the procedures that have been tested.

As the field collecting stage is a holding operation, an *in vitro* laboratory will be needed both before and after the collecting mission. As much as possible should be done in advance (e.g. preparation of culture medium, labelling of vessels, cleaning and sterilization of instruments), and as much as possible deferred to the receiving laboratory (e.g. resterilization of inoculated tissue, transfer of tissue to complex media).

Back at the laboratory

At the receiving laboratory, each type of explant will need to be handled by a different procedure to maximize its chances of survival. The other crucial variable is the destination of the material. Disease eradication, indexing and quarantine stages may need to be integrated into the process. Decisions will thus have to be made at the planning stage about how the material collected will be handled at the receiving institute. The important questions that need to be asked – and answered – are as follows.

Can the culture simply be placed in a controlled environment room
or, alternatively, does it require resterilization and further dissection
to yield an explant free of contamination and suitable for culture?

Obvious examples of the latter situation would be coconut zygotic embryos enclosed in an endosperm plug that need to be resterilized and dissected. Seeds might require resterilization and probably, since they are likely to be immature if selected for *in vitro* collecting, dissection to extract the embryo or embryo axis for rescue *in vitro*. For shoots, particularly woody ones, resterilization and dissection for grafting *ex vitro* or *in vitro* may be relevant. In the case of the *in vitro* option, this might be a method similar to conventional bud grafting but using an *in vitro* germinated rootstock or it might involve micrografting, which can

be used to eliminate viruses (Navarro, 1981; Kartha, 1986). V.M. Villalobos (pers. comm.) and L.A. Withers (unpublished) have successfully grafted *in vitro* cacao shoots from somatic embryos and axillary buds from woody shoots respectively on to seedlings.

What type of culture is relevant to the intended destination of the material?

If the priority is to get the germplasm into storage under conventional conditions in a field gene bank or to produce seeds for storage in a seed gene bank, it is logical to produce an independently growing plant by the most direct route possible. Clearly, use of a particular *in vitro* collecting method will depend on the existence of a successful method of regenerating plants from explants. Information on this will have to be sought on a species-by-species basis from the relevant literature. If the material is so limited in quantity that it would be a good idea to multiply it before transfer to conventional conservation in the field or seed gene bank, or if its destination is the *in vitro* gene bank, then propagation *in vitro* to produce several plants should be considered.

For most species with orthodox seed, the conventional seed gene bank holding seeds at low moisture content and low temperature will be the eventual destination. This storage method is technically uncomplicated and offers a high degree of security (Justice and Bass, 1978; Ellis *et al.*, 1985). For recalcitrant seed, conservation is more problematic (Chin and Pritchard, 1988). Most such species are currently conserved in field gene banks, but new *in vitro* conservation methods, particularly cryopreservation (storage in liquid nitrogen), may offer a viable alternative in the near future. These alternatives are very welcome as the field gene bank is a costly and risky approach to conservation, the germplasm being exposed to loss by weather damage, disease and neglect (Withers, 1989; Withers and Engels, 1990).

For material unable to produce seeds because it is sterile (e.g. *Musa*) or for clones for which seed storage would break up valuable gene combinations (e.g. root and tuber crops, many fruit trees), storage in field gene banks is also the most common conservation method. However, *in vitro* conservation by cryopreservation for the long term or slow growth for the short to medium term again promises to provide a safer alternative. More details of *in vitro* storage methods are given by Withers (1980, 1985a,b, 1987b, 1992), De Langhe (1984) and Dodds (1991).

Given that the decision has been made to multiply the material *in vitro* before regenerating plants, what is the most suitable propagation system?

Generally speaking, non-adventitious systems offer greater genetic stability. Therefore, preference should be given to simple nodal cutting methods such as are common in potato (Espinoza *et al.*, 1984), cassava (Roca, 1984) and some woody species, or to multiple shoot formation, as

used for some ornamentals and temperate fruits (Conger, 1981; Tisserat, 1985). Where this approach is impossible or impractical due to the nature of the explant and/or the state of propagation technology for the species in question, the next most suitable options of direct adventitious shoot formation or embryogenesis should be taken. These would be the likely routes for *Musa* shoot explants (Vuylsteke, 1989) or immature zygotic embryos of cacao (Pence *et al.*, 1980) respectively. The least suitable system would be one involving callus formation and indirect, adventitious regeneration (Scowcroft, 1984). Callus should only be used if no other option is available. However, some of the most suitable culture systems from the point of view of genetic stability may not be very amenable to *in vitro* conservation. Thus it may be necessary to compromise and choose a less suitable culture system so that the security of *in vitro* conservation can be exploited.

A further point to consider relates to the maintenance of genetic diversity. The basic objective of germplasm collecting is to acquire representative genetic diversity of the target gene pool from the target region. This should not be forgotten in the handling of the germplasm after it has been collected. Just as the collecting strategy should be designed to sample the maximum level of genetic diversity, the plant regeneration and/or multiplication strategy should be designed to maintain that level of genetic diversity. Thus, clonal propagation *in vitro* should be used if it is necessary to bulk up a rare genotype, but not to multiply one genotype at the expense of others. The plant regeneration procedures used should be widely applicable across the range of genotypes involved, so that propagation does not act as a genetic 'filter'.

Examples of *in vitro* collecting

It cannot be emphasized too strongly that the great advantage of *in vitro* collecting is its adaptability and flexibility. For this reason, no hard-and-fast rules or recipes are offered, only general guidelines to help in the adaptation of the concept to new species and situations. The purpose of this section is to present a number of examples, some published and some only reported informally, that illustrate the scope of the approach and how it could assist germplasm collecting missions (see also Withers, 1987a).

Theobroma cacao

One of the earliest species to be explored was cacao, for which an attempt was made to find an alternative to transporting budwood sticks from the collecting site to the nursery. Experiments by Yidana and colleagues (Yidana *et al.*, 1987; Yidana, 1988) demonstrated that a sterilization procedure for nodal stem segments using drinking-water purifying tablets (containing the active ingredient 'Halozone' (*p*-carboxybenzenesulphondichloroamide) at a concentration of $0.4\,g\,l^{-1}$) and an

agricultural fungicide (e.g. FBC Protectant Fungicide at 0.05%) was effective without subsequent washing.

Inoculation on to semisolid medium containing fungicide (e.g. Tilt MBC at 0.1%), with or without antibiotics (e.g. rifamycin plus trimethoprim, each at 30 mg l^{-1}), would maintain the tissues in a relatively clean, although not absolutely contaminant-free, condition for up to six weeks. (Optimization of sterilants and culture media was carried out using a leaf disc assay system.) Shoot outgrowth and occasional rooting were reported. The field equipment was minimal, consisting of racks of plastic tubes of culture medium prepared in advance in the laboratory, jars of boiled water, preweighed/precounted sterilants, plastic forceps, and scissors/secateurs. Inoculation was carried out in the open air. The major limitation of this protocol was the lack of a good *in vitro* propagation method to process the material collected. However, it does illustrate the extent to which the rules of *in vitro* culture can be stretched.

Gossypium

Collecting germplasm of cotton and its wild relatives can be hindered by the unpredictable availability of viable seeds. Accordingly, Altman *et al.* (1990) attempted to develop an *in vitro* method for use in the field in Mexico. Stem nodal cuttings were surface-sterilized with 20% commercial bleach in 30% ethanol for 45 seconds and then inoculated directly into a medium containing half-strength salts, 1% glucose, the antibiotics rifamycin and trimethoprim each at 15 mg l^{-1}, 1 g l^{-1} of the fungicide Tilt MBC, 1 mg l^{-1} naphthalenacetic acid, 0.5 g l^{-1} casein hydrolysate and 9 g l^{-1} agar. No special work bench or other protective environment was used, transfers being made in the open air. After being in transit for up to three weeks, the cuttings were resterilized with 4% bleach solution, treated with rooting hormone and planted in a sterile soil/sand/vermiculite mix supplemented with lime and slow-release nutrients.

Although it was clear that the collecting stage itself was successful, difficulties were experienced in rooting and raising plants from the cuttings. This again emphasizes the importance of developing or adapting a method for processing the collected material.

Forage grasses

Forage grasses share many of the collecting problems of cacao and cotton, as well as the risk of grazing by animals, but they are structurally very different. Thus, in the case of the grasses *Digitaria* and *Cynodon*, the available explant was a herbaceous plantlet. Despite the less robust nature of this material and its different *in vitro* culture requirements, it could be treated similarly to woody material. Ruredzo (1989) used a simple method similar to that of Yidana for cacao. In this case, the collecting site was sufficiently close to the collector's base (a hotel) for the inoculation to be carried out there. In such circumstances, it would be unnecessarily demanding to carry out the inoculation in the field.

Cocos nucifera

The final example in this brief survey of model plant systems and technical approaches is coconut. Two main points are important here. Firstly, coconuts are bulky and heavy, making them costly to transport. Secondly, coconut seeds are recalcitrant. Accordingly, several workers have adapted *in vitro* embryo culture techniques to the field collecting of coconut germplasm.

In all cases, the basic sequence of operations is as follows:

- dehusking and cracking open the nut;
- extracting a plug of endosperm containing the embryo using a cork borer;
- dissecting the embryo;
- inoculating into culture.

The differences between the various approaches lie in the degree to which an attempt is made to reproduce laboratory conditions in the field, the amount of culture work actually carried out in the field as opposed to the recipient laboratory and, consequently, the point at which sterilization is carried out.

The simplest method is that employed by Rillo and colleagues in the Philippines (E.P. Rillo, pers. comm.). The endosperm plugs were transported from the field in refrigerated plastic bags containing coconut water collected from some of the opened nuts. Sterilization and inoculation were carried out in the recipient laboratory (Rillo and Paloma, 1990). A simple insulated container kept the endosperm plugs at an adequately stable temperature in transit. The field requirements were minimal: implements to dehusk and open the nuts, cork borers, plastic bags, carrying containers. Personnel training was also undemanding. All that was required in addition to the normal collecting skills was the ability to recognize the position of the live embryo and extract it in the endosperm plug without damage. Instruction in this technique was included in a training course cosponsored by IBPGR and the Philippines Coconut Authority (PCA) in 1990. Students very quickly and easily learned the embryo extraction and inoculation process.

More complex protocols were used by Assy Bah *et al.* (1987) and Luntungan and colleagues (H. Luntungan and J.S. Tahardi, pers. comm.) in Côte d'Ivoire and Indonesia, respectively. They carried out surface sterilization of the endosperm plugs in the field using calcium hypochlorite at $45\,g\,l^{-1}$ and inoculation into a simple salt solution (KCl at $16.2\,g\,l^{-1}$) for transport back to the laboratory, where a second sterilization could be carried out if necessary, followed by dissection and inoculation of the embryo onto standard culture medium (Assy Bah *et al.*, 1989).

The field equipment requirements were more complex than in the first example. It was necessary to provide sterilants, sterile water for washing, containers of salt solution, a burner, a spirit lamp to sterilize instruments and flame openings of containers, forceps and a simple

workbench. More training in the appropriate manipulations was also required. Assay Bah and colleagues also carried out direct inoculation into culture in the field. This obviously required more manipulative skills, greater care in handling the explants and a more protective working environment, such as an upturned box to keep out airborne contaminants.

The techniques described above accept the limitations of working in the field and take a relatively low-technology approach to solving them. The method of Sossou *et al.* (1987) follows a very different logic. In this case, every effort was made to overcome the inadequacies of the field environment and provide an inoculation facility that was almost as sophisticated as the laboratory. An inflatable glove box which could be sterilized with alcohol was used and the inoculation procedure was exactly as would be carried out in the laboratory, requiring the same level of manipulative skills. The glove box, pump, instruments, lamp, solutions and culture vessels all had to be transported with care to and from the collecting site.

In coconut, collecting, embryo culture, establishment of *in vitro*-germinated embryos in the field and germplasm distribution *in vitro* are all well developed (Assy Bah *et al.*, 1987, 1989). However, reproducible clonal multiplication techniques are not yet available. Somatic embryogenesis would appear to be the most likely approach. Zygotic embryos can be maintained for up to a year in slow growth but shoot or somatic embryo cultures would be preferable for this method. Cryopreservation of zygotic embryos, although at an early stage of development, appears promising (Assy Bah and Engelmann, 1992a,b). The model of oil-palm (Engelmann *et al.*, 1985) would suggest that, once a suitable somatic embryo system has been developed, its cryopreservation should be attainable.

Other species

All experience points to the *in vitro* collecting approach being widely applicable and easily adapted. In an IBPGR-sponsored training course held at the Centro Agronémico Tropical de Investigaçión y Enseñanza (CATIE), Costa Rica, in 1990, students experimented with adapting the basic methodology to a wide range of species. These included banana, coffee and citrus, all of which were successfully established in culture via either vegetative tissues or embryos. Methods for *Prunus* spp. and *Vitis* spp. based on the approach used for cacao described above have been developed (Elias, 1988).

Examples involving various root and tuber crops are briefly described in Chapters 21 and 32. In cassava, there is a well-established methodology for all stages of conservation from collecting to slow-growth storage and distribution, including, for example, the clonal propagation stage that is a problem with coconut. IBPGR (and now the International Plant Genetic Resources Institute (IPGRI)) and the Centro Internacional de Agricultura Tropical (CIAT) have been collaborating since 1987 to develop and test management procedures for

in vitro active gene banks using cassava as a model (Chavez *et al.*, 1987). One hundred genotypes selected from CIAT's field gene bank have been introduced into culture via disease eradication and indexing and transferred to slow-growth storage. Characterization using morphological descriptors has been supplemented by isozyme analysis and these data are being used as standards against which to assess the stability of germplasm recovered from the gene bank for periodic monitoring.

Conclusions

These examples illustrate the flexibility of *in vitro* collecting. They involve different levels of complexity at the field stage and a range of explants suitable for different species. The spectrum of examples is given as a stimulus to the imagination rather than for exact adoption. In fact, the work of Sossou *et al.* (1987) with coconut, for example, is probably unnecessarily complex. It is important to judge very carefully the need for such complicated procedures because simpler alternatives, in this case coconut embryos enclosed in endosperm plugs, may be perfectly adequate. It should be carefully considered whether ingenious but intricate procedures are really necessary, whether they are compatible with other tasks to be accomplished on the collecting mission and whether they would, by their nature, place too great a burden on transport, training and technical backup.

Thus, the important message is that the operations carried out in the field should be only those that absolutely must be carried out there. These operations will be defined by the condition of the plant material, the nature of the collecting environment and the duration of the journey back to the laboratory. Any other operations should be delayed until the collected material has reached the laboratory.

The objective of this chapter has been to set the scene by showing how some of the problems of germplasm collecting could be overcome by the imaginative application of *in vitro* procedures. It is emphasized that this new approach to collecting is technically unsophisticated but cannot be undertaken without adequate background knowledge, preparation and planning, because it must be seen as part of a comprehensive conservation scheme that flows from quarantine and disease indexing to storage, distribution and use. *In vitro* collecting is not a means of circumventing quarantine and disease indexing procedures: it may reduce the risk of introducing pests and diseases (IBPGR, 1988) but this does not lessen the need for vigilance and the need to comply with appropriate phytosanitary procedures and regulations. After processing through quarantine, disease indexing and disease eradication procedures, the collected germplasm should be multiplied in preparation for storage. There must be a plan to store the germplasm safely by slow growth (short- to medium-term, active conservation) or cryopreservation (long-term, base conservation), making it available for use when necessary and in

adequate quantities. For material stored *in vitro* to be used, plants must be regenerated: it would be useless to collect *in vitro* if no method of recovering plants were available. The material must also be distributed. It is logical to distribute *in vitro* conserved germplasm in the form of a culture and this can now be carried out for many species. In fact, the international distribution of clonal crop germplasm in the form of *in vitro* cultures from several centres of the Consultative Group on International Agricultural Research (CGIAR) is now routine (Espinoza *et al.*, 1984; Roca *et al.*, 1984; Ng and Hahn, 1985; Withers and Williams, 1985; Schoofs, 1991). *In vitro* collecting will only achieve its full potential if integrated into such a comprehensive system, and adequate planning is therefore critical.

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