TISSUE CULTURE FOR CONSERVATION OF PERENNIAL CROPS

Oradee Sahavacharin[1]

Southeast Asia is the centre of diver sity of large numbers of perennial crops. These are root and tuber crops which in general can feed two time more people from a unit area than cereals, and the nutritional value of sweet potato, yam and other root crops is not less than that of cereals, which can be compensated by side dishes in the case of cassava. Among the strategies suggested for improving the root and tuber crops, and increasing their production the conservation, evaluation, and exchange of genetic resources are most important. Many tropical fruit crops also have their home in Southeast Asia, but no one nation has as yet considered them as highly important. Therefore, their development is somewhat neglected. Under an ASEAN programme, post-harvest technology of fruits received attention, but pre-harvest technology such as quick propagation and breeding has yet to be considered.

Although germplasm collections have always been an essential part of plant breeding work, it has until recently been possible to rely upon the availability of further genetic diversity in primitive crop varieties and related wild species. It is now recognized that these genetic resources are under considerable threat as a result of changes in agricultural practices that have already led to the replacement of many of these varieties by modern ones with much restricted genetic diversity. When these losses are associated with changes in the natural habitats in which related wild species have hitherto survived, there is a serious danger of future plant breeding programmes being impeded by the shrinking genetic bases of some crops. This danger was recognized in 1974 when the International Board for Plant Genetic Resources (IBPGR) was established. The basic function of the IBPGR is to promote an international network of genetic resources centres to further the collection, conservation, documentation, evaluation and the use of plant germplasm.

The size of the task facing genetic resources centres is enormous. However, it is fortunate that the genetic diversity of many crop species can be satisfactorily conserved in the form of seed which can be stored in a relatively small space for many years.

Seed storage, however, is not suitable for all crops. A further problem in conservation is that of seed recalcitrance. Seeds which lose viability when dried below a certain water content or when exposed to low temperatures (occasionally well above zero) are described as *recalcitrant*, distinguishing them from *orthodox* seeds which can be stored at a low temperature (ca-20° C or lower) and low moisture content. Among the plants with seed showing this type of behavior are many timber, food and other commercial crops exemplified by *Juglans spp.* (walnuts), *Quercus spp.* (caks), *Coffea spp.* (coffee), *Hevea brasiliensis* (rubber

[1]Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkhen, Bangkok, Thailand

tree), and *Elaeis guineensis* (oil palm). Several categories of plants present serious conservation problems since their germplasm cannot be stored by conventional seed storage techniques. There will always be a need to store vegetative material where seed is not produced, or where the seed is not suitable for long-term storage or if there is a need to conserve exact genotypes. Vegetative parts af tubers, roots, cuttings etc. must be stored or maintained in the field which can be expensive. Furthermore, they can be destroyed or lost by pathogens, natural diasters, human errors, etc.

Among the vegtatively propagated crops in this category are *Colocasia esculenta* (taro), *Dioscorea spp.* (yams), *Ipomoea batatas* (sweet potato), *Manihot spp.* (cassavas), *Musa spp.* (bananas), *Solanum spp.* (potatoes), and *Xanthosoma sagittifolium* (cocoyam).

The scale of this problem can well be illustrated by considering the case of potato germplasm. The germplasm collection for this genus, acquired at the Centro International de la Papa, Peru, through exploration in South America, grew from 4,000 accessions in 1975 to an anticipated and realized 12,000 in 1980. Among the accessions are some infertile triploid and pentaploid cultivars for which seed storage would be impossible even if desirable.

Another important group of plants includes those which can be propagated by seed but which would profit from a vegetative means of propagation in order ot increase stocks of valuable F_1 hybrids or high yielding and disease resistant genotypes. Palms including *Cocos nucifera* (coconut palm),*Elaeis quineensis*(oilpalm) and *Phoenix dactylifera* (date palm), for which no useful method of vegetative reproduction exists would benefit greatly from the development of both methods for clonal propagation and for propagule storage.

Many long lived forest trees incluading Angiosperms and Gymnosperms do not produce seed until a certain age, and must be propagated vegetatively when it is desired to propagate parent rather than progeny.

As mentioned earlier, storing vegetative parts or maintaining them in the field is expensive or problematic because of pathogens, climatic perturbations, other natural diasters, and human error. Alternative methods must be sought. In order to resolve these problems in conservation the possibility can be entertained of introducing the explants from the vegetatively propagated crop plants, mature perennials and plants with recalcitrant seed into tissue culture and storing their germplasm. This is where *in vitro* methods could be extremely valuable for the long-term storage of germplasm because large numbers of clones can be stored in a relatively small space, in a disease-free state and it is convenient for the international dissemination of such material.

The blanket term 'tissue culture' colloquially covers a wide range of techniques including *in vitro* culture of organs, (shoot tips, root tips, flowers, ovaries, ovules, embryos, anthers etc.) tissues, cells, and protoplasts. In 1902, Haberlandt published on his unsuccessful but pioneering attempt to cultivate cells from leaves of several angiosperms. Twenty eight years later, plant tissue culture became available with the successful

research efforts of White in the U.S.A. and Gautheret in France in about 1930. For the past 50 years plant tissue culture has been established as a potential research tool and an economical agricultural practice.

For simplification and convenience the major economic applications for plant tissue culture can be placed in these categories.

1. Production of pharmaceuticals and other constituents. Although more than 20 years of research have been devoted to achieving the feasibility of cell cultures as sources of plant constituents, only now are economic benefits being realized. Some constituents currently produced commercially include, camptothecin, protenase inhibitors, anti-viral substances and antibiotics.

2. Establishment of pathogen-free stocks. Any cultivar that has been propagated for substantial periods by traditional asexual methods should be suspected of carrying one or more virus or virus-like pathogens. Pathogens of this nature can be eliminated by culturing shoot tips and a virus-free stock can be re-established.

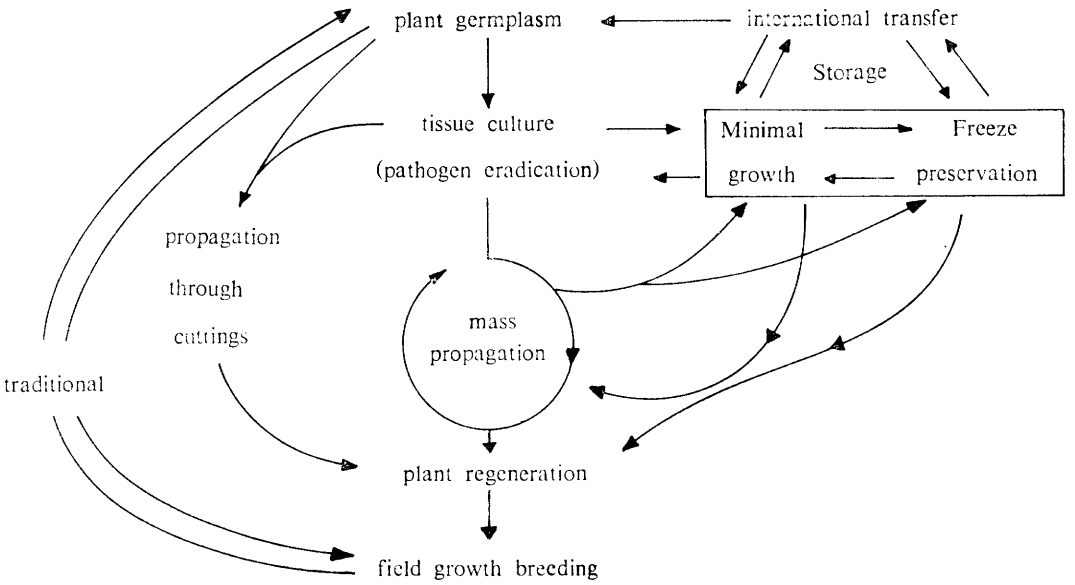
3. Rapid clonal propagation. The most extensive commercial use of plant tissue culture has been in clonal propagation of orchids ferns, indoor foliage plants, certain woody ornamentals, diverse bulbs, and other food crops including potato, pineapple, banana and strawberry.

4. Genetics and plant breeding. This procedure is currently or potentially applicable to various genetic investigations. Some are intended simply to enhance the traditional plant breeding practice, e.g. embryo, ovary, and ovule cultures. Cell cultures could be used in mutant induction and isolation. Anther or microspore culture should be helpful in mutagenesis and in obtaining homozygous diploids. Protoplasts will enable hybridization by fusion between somatic cells of selected cultivars. Furthermore, it is hoped that gene transfer among higher plants will be achievable some day through the mediation of protoplasts. The transfer might require plasmids, viruses, or other agents or gene carriers.

The economic benefits that could result from culturing the tissues of important perennial crops are just beginning to be appreciated, and the best methods for doing this have not yet been fully worked out. Withers (1980) developed the following general scheme for the storage of genetic material, and it appears to be a good model for the storage of germplasm from important perennial crops.

This scheme is proposed for the incorporation of the two favoured storage methods: minimal growth and freeze-preservation into a genetic conservation programme. Ideally, the two systems should be able to interchange material and provide ready supplies for field growth, breeding and dissemination. Clonal mass propagation is likely to become of increasing importance in the future, permitting multiplication of accessions for utilization and exchange and also for the storage of replicates.

As soon as *in vitro* methods are discussed in the context of germplasm storage, either in a growing state (minimal growth) or in suspended animation (freeze-preservation), the much-publicized problems of genetic instability and loss of morphogenetic potential must be



carefully examined with a view to selecting a satisfactory culture system. The incidence of genetic variability deriving from mixoploidy in the original explant (such as *Saccharum officinalis* which is is known as chromosome mosaics), and from events occuring during culture initiation and maintenance can sometimes be a problem. There is evidence that cultures may be stabilized by careful choice of inoculum and care in maintenance. Genetic variation may increase with time in growing cultures. Callus cultures are generally initiated from non-meristematic tissues which are polysomatic in many angiosperm families. There is the possibility that from the outset they might not be representative of the normal genetic constitution of the parent plant. It would, therefore, seem that callus cultures should not, at present, be considered for germplasm storage except in those species in which long-term genetic stability and retention of morphogenetic potential has been satisfactorily demonstrated.

Henshaw (1979) reported that liquid nitrogen freeze-storage procedures are often advocated for germplasm storage but their use with callus or suspension culture is likely to be unsatisfactory for this purpose if there is the possibility of genetic changes in the cultures during the growth phases before and after storage. Moreover, even if a satisfactory freeze-storage procedure were available, the possibility of selection of aberrant cell types in the explant would not be avoided. In view of these problems with callus culture it is obvious that, where possible, alternative *in vitro* methods should be assessed for germplasm storage. Fortunately experience from the commercial use of shoot-tip cultures for the propagation of ornamentals suggests that such cultures, particularly those

based on non-adventitious shoot production, usually retain the considerable genetic stability characteristic of the shoot meristem *in vitro*. The fact that shoot-tip cultures have already proved their worth commercially, both for rapid clonal propagation and for the eradication of systemic pathogens, including viruses strengthens the argument that they could also be used for germplasm storage. There is the practical advantage of use of common facilities, and in addition, the potential for rapid propagation is highly desirable when garmplasm is to be taken from storage. Further, the value of that germplasm would be enhanced if it were to be available in a disease-free state.

If shoot-tip cultures are to be used on any scale for germplasm storage, attention must be paid to the requirements for initiation of cultures, storage conditions and the maintenance of genetic stability. In a comprehensive germplasm collection a single species might be represented by a large number of genotypes which could have different medium requirements. It is important, therefore, to establish a routine by which these variations can be readily identified. Generally speaking, the inorganic nutrient requirements of cultures used for propagation purposes seem to be remarkably uniform. Murashige and Skoog's media, Knudson's media as well as White's media were generally effective. Some adjustments of organic nutrients, especially the nitrogenous compounds, might be important, however within a species or genus, fine adjustments of the hormone levels to induce optimal shoot and root development could be all that is necessary.

The optimal conditions for culture initiation and for rapid propagation are unlikely to be the same as those for long-term storage of cultures where minimal attention and genetic stability must be major considerations. Both purposes are likely to be served by reduced growth rates. The ultimate possibly being achieved by freeze preservation. The standard culture media have all resulted from efforts to achieve fast growth rates or high yields. Very little attention has been given to the problem of devising conditions which will keep cells alive for long periods in a non-dividing or slowly dividing state. Although such conditions clearly prevail *in vivo* and are probably an essential component of the mechanisms controlling differentiation, little is known about their nature, and attempts to achieve the same state *in vitro* can often lead to cell senescence and death.

At present, liquid nitrogen freeze-preservation is probably the only feasible method for long-term storage of cultures in a completely non-dividing state. Unfortunately most of the reports of successful freeze-preservation of cultures have been concerned with callus or suspension cultures and, for reasons given earlier, these may not be appropriate for germplasm storage. There have now, however, been a few reports of the successful freeze-storage of shoot meristems in carnation, tomato and potato. A common feature of all these reports is that the shoot tips were treated with a cryoprotectant, then frozen at higher cooling rates than have generally been successful with callus or suspension cultures. Whether this approach will prove to be the most suitable for

general use with meristems remains to be seen but clearly it is now possible to contemplate the use of freeze-preservation techniques with shoot-tip cultures for germplasm storage thus avoiding the problems associated with the use of callus of suspension cultures. Further work is required to improve the successful rate with these techniques and to demonstrate whether they are more widely applicable.

The advantage of using freeze-preservation techniques for germplasm storage is that, once the material has been successfully frozen, little further attention should be required except to ensure that the liquid nitrogen supply does not fail. Furthermore, evidence from work with animal colls suggests that there should be a high level of genetic stability, the limiting factor being the damage that occurs as a result of ionizing radiations which might only become significant after decades. These techniques would not, however, completely replace procedures which maintain suitable cultures under conditions only allowing minimal rates of growth. The advantages of the latter procedure are that the stock is constantly being replinished. It can generally be seen to be alive and is available for almost immediate distribution.

There are number of possible approaches to the problem of achieving minimal growth rates for the purpose of increasing the longevity of culture. Basically, they either utilize specific growth inhibitors (e.g. abscisic acid) or conditions which have more general effects on metabolic rates (e.g. temperature, gaseous conditions, nutrient availability, osmotic stress). Once satisfactory growth-limiting conditions have been established, longevity might be further increased by ensuring that other factors do not become depleted to the point that senescence is induced.

The following results indicate that it should be possible to devise conditions which will reduce the growth rates of shoot-tip cultures to such an extent that they can survive for at least one year before requiring transfer to fresh medium. Grape shoot-tip cultures stored at 9° C required only an annual transfer to fresh medium. Virus-free cultures of strawberry could be stored for up to six years at 4° C in the dark if one or two drops of medium were added to the culture every three months to replenish the nutrients and replace the water lost by evaporation. Investigations with a range of potato cultivars and species indicated that reduced tempertures in the range of 6-10° C substantially increased the longevity of cultures. Improvement could also be obtained by the addition of an inhibitor, such as abscisic acid, to the medium by the use of sufficiently low sucrose concentrations to retard growth by the induction of osmotic stress. Alternatively, higher sucrose concentrations (8 %) than normal (3 %) in the medium will further increase the longevity of cultures whose growth has been ratarded by lower temperatures.

Accepting, therefore, that *in vitro* methods of germplasm storage are likely to be practicable for many vegetatively propagated crop species. Can it be concluded that they would be economically advantageous? This question can only be answered in relation to local circumstances, undoubtedly there will be germplasm collections in which the

storage of vegetative material presents few problems perhaps because of the scale of the operation, the absence of serious pathogens, the lack of natural hazards, the cheapness of labour, the longevity of plants in the field, etc. In those cases, field plantations will continue to be the preferred method of conservations. Other collections, however, can be very costly particularly if there are serious pathogen problems associated with a need for the international distribution of the material or if rapid production of plants from clones stored in the collection is important. In these circumstances there can be real advantage in using *in vitro* methods to reduce dependence on traditional procedures, perhaps by forming a reserve disease-free collection of the more valuable material. It is for this reason that the International Potato Centre, Peru, which has an international responsibi lity for the storage and utilization of potato germplasm, extensively utilizes shoot-tip culture techniques.

Generally speaking, pathogen elemination is likely to be the key issue that will influence a decision to adopt *in vitro* methods of germplasm storage. For many crops, the cost of investment in reliable facilities for pathogen testing and elimination would be very high. Since *in vitro* methodology is likely to be involved in any such procedure, the extra cost of facilities for storage, propagation and international distribution of disease-free germplasm would, in comparison, be modest. Alternatively, if the cost of producing disease-free germplasm are to be borne by a centralized facility, government or business, the establishment of a local facility for *in vitro* germplasm storage could well be advantageous, since these methods probably offer the most satisfactory means of preventing the re-infection of stocks (Henshaw, 1979)

In conclusion, the conservation of plant genetic resources is one of the most important and urgent tasks facing plant scientists today, and this need is greatest in the tropics where plant diversity is great and where many species are being destroyed. Tissue culture techniques have now been developed that allow scientists to preserve the genetic diversity of many species. Every possible effort should therefore be made to identify and collect samples of all the wellknown fruit crops such as mango, durian, rambutan, lansium and jackfruit and perennial crops such as yams, cassava, and taro and the lesser-known species such as some Zingiberaceae that may prove to be economically important in the future.

In Thailand during the past year collections of tropical fruits, banana varieties, edible legumes, *Momordica sp*, and a number of medicinal plants have been made. It is also hoped that a centre for the storage of tissue cultures of perennial crops can be established here in the near future that will allow Thailand to effectively preserve a large part of its rich plant genetic resources I hope that plant scientists, government officials, and businessman can cooperate to see this goal realized here in Thailand as well as in the other nations of Southeast Asia

REFERENCES

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