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Plant conservation and use of biotechnology

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ABSTRACT: Conservation and sustainable use of genetic resources is essential to meet the demand for future food security. Advances in biotechnology have generated new opportunities for genetic resources conservation and utilization. Techniques like *in vitro* culture and cryopreservation have made it easy to collect and conserve genetic resources, especially of species that are difficult to conserve as seeds. While technologies like enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) have provided tools that are more sensitive and pathogen specific for seed health testing, tissue culture methods are now widely applied for elimination of systemic diseases such as viruses for safe exchange of germplasm. Molecular markers are increasingly used for screening of germplasm to study genetic diversity, identify redundancies in the collections, test accession stability and integrity, and resolve taxonomic relationships. The technology is also expanding the scope of genetic resources utilization.

***Keywords*:** Plant conservation, Biotechnology, genetic resources

INTRODUCTION

A large number of crop species have seeds, which are termed orthodox, i.e. that can be dehydrated down to low water contents and can thus be stored at low temperature for extended periods (Roberts, 1973). There are three main categories of plant species for which conservation in seed form is problematic. First, some plants such as banana and plantain do not produce seeds and are thus propagated vegetatively.

Second, some species such as potato or sugarcane include both sterile genotypes and genotypes which produce orthodox seeds. However, these seeds are generally highly heterozygous and are thus of limited interest for the conservation of particular genotypes. These species are thus mainly maintained as clones. Third, numerous fruit and forest tree species, especially from tropical origin, produce recalcitrant seeds, i.e. seeds that cannot be dried to sufficiently low moisture level to allow their storage at low temperature (Roberts, 1973). There is also a large number of species, termed intermediate (Ellis et al., 1991) for which conservation in seed form is still problematic. The traditional ex situ conservation method for these categories of plant species is in the form of field collections. Conservation in the field presents major drawbacks, which limit its efficacy and threaten the safety of plant genetic resources conserved in this way.

Until now, most activities on ex situ conservation of plant biodiversity have focussed on crop species. However, conservation of wild, rare and endangered plant species has also become an issue of concern. Indeed, as highlighted by Sarasan et al. (2006), the world's biodiversity is declining at an unprecedented rate. During the period 1996–2004, a total of 8,321 plant species have been added to the Red List of Threatened Species (IUCN, 2004), and the number of plants recorded as critically endangered has increased by 60%. In the case of wild species, the traditional conservation approach is in situ conservation. However, it is now recognised that ex situ techniques can be efficiently used to complement in situ methods, and they may represent the only option for conserving certain highly endangered and rare species (Ramsay et al., 2000). It is therefore of paramount importance to develop techniques ensuring optimal storage and rapid multiplication of such species.

Botanic gardens play a very important role in ex situ conservation of plant biodiversity. UNEP (1995) estimated that botanic gardens conserve more than one third of the world's flowering plants, among which Botanic Gardens Conservation International identified more than 15,000 threatened species. Botanic gardens and agricultural genebanks should be seen as playing a complementary role for the conservation of plant biodiversity (Engels and Engelmann, 1998).

The development of biotechnology leads to the production of a new category of germplasm including clones obtained from elite genotypes, cell lines with special attributes and genetically transformed material (Engelmann, 1992). This new germplasm is often of high added value and very difficult to produce. The development of efficient techniques to ensure its safe conservation is therefore of paramount importance. In the light of the problems presented by the different categories of plant species outlined above, it is not surprising that efforts have been made to improve the quality and security of conservation offered by field genebanks and botanic gardens and to understand and overcome seed recalcitrance to make seed storage more widely available. However, it is clear that alternative approaches to genetic conservation are needed for these problem materials, and since the early 1970s, attention has turned to the possibilities offered by biotechnology, specifically in vitro or tissue cultures.

Advances in biotechnology, especially in the area of *in vitro* culture techniques and molecular biology provide some important tools for improved conservation and management of plant genetic resources. Ramanatha Rao and Riley (1994) comprehensively reviewed the role of biotechnology and the ways in which some of the available technologies assisted in carrying out PGR activities more effectively. Therefore, the current study was carried out to study the plant conservation and use of biotechnology.

Applications of biotechnologies for conservation

In vitro collecting. Collectors are faced with various problems when collecting germplasm of recalcitrant seed and vegetatively propagated plant species. Collecting missions often require travelling for relatively long periods in remote areas. It is thus necessary to keep the material collected in good state for some d/wk before it can be placed in optimal growth or storage conditions. There are thus great risks that recalcitrant seeds either germinate or deteriorate before they are brought back to the genebank or botanic garden. In addition, many recalcitrant seeds have a sheer weight and bulk, which is a source of problems in terms of volume of material to handle and which induces additional costs, if an adequate sample of the population is to be collected. With vegetatively propagated species, the material collected will consist of stakes, pieces of budwood, tubers, corms or suckers. Not only will most of these explants not be adapted to survival once excised from the parent plant but they will also present health risks due to their vegetative nature and contamination with soil-borne pathogens (Withers, 1987).

Difficulties can also be encountered when collecting germplasm of orthodox seedproducing species. Even with careful planning of the time of the collecting mission, there might be no or little seed available for all or part of the germplasm to be collected, or seeds might not be at the optimal developmental stage, shed from the plant or eaten by grazing animals (Guarino et al., 1995). These problems can be overcome if it is realized that the seed is not the only material which can be collected: Zygotic embryos or vegetative tissues such as pieces of budwood, shoots, apices or even leaf discs can be sampled, transported and grown successfully if placed under adequate conditions. Following an expert meeting organised by IBPGR in 1984 and sponsorship of various research programmes, simple and efficient in vitro collecting techniques have been developed for different materials including embryos or vegetative tissues of various species including crops such as coconut or cacao, as well as wild and endangered species (Pence et al., 2002). The critical points to consider for the development of in vitro collecting techniques have been synthesized and analysed by Withers (1995).

Slow growth storage

Growth reduction is generally achieved by modifying the environmental conditions and/or the culture medium. The most widely applied technique is temperature reduction, which can be combined with a decrease in light intensity or culture in the dark. Tropical species are often cold-sensitive and have to be stored at higher temperatures, which depend on the cold sensitivity of the species. Musa in vitro plants can be stored at 15°C without transfer for up to 15 mo (Banerjee and De Langhe, 1985). Other tropical species such as cassava are much more cold-sensitive since cassava shoot cultures have to be conserved at temperatures higher than 20°C (Roca et al., 1984). Modifications of the culture medium can include dilution of mineral elements, reduction of sugar concentration, changes in the nature and/or concentration of growth regulators and addition of osmotically active compounds.

Numerous parameters influence the efficiency of in vitro slow growth storage protocols including the type of explants, their physiological state when entering storage, the type of culture vessel, its volume and the volume as

well as the type of closure of the culture vessel (Engelmann, 1991). In vitro slow growth storage techniques are being routinely used for medium-term conservation of numerous species, both from temperate and tropical origin, including crop plants, e.g. potato, Musa, yam, cassava (Ashmore, 1997; Razdan and Cocking, 1997; Engelmann, 1999) and rare and endangered species (Fay, 1992; Sarasan et al., 2006). However, if in vitro conservation appears as a simple and practical option for long-term conservation of numerous species and has obvious wide medium-term applications, its implementation still needs customizing to any new material, continuous inputs are required and long-term questions remain as regards the genetic stability of the stored material.

Moreover, it is not always possible to apply one single protocol for conserving genetically diverse material. As an example, a storage experiment performed with an in vitro collection of African coffee germplasm including 21 diversity groups revealed a large variability in the response of the diversity groups to the storage conditions (Dussert et al., 1997). Some groups showed high genetic erosion during storage whilst others did not show any erosion. Technical guidelines have been published recently (Reed et al., 2004), which provide guidance to researchers and genebank and botanic garden managers for the establishment and management of in vitro germplasm collections.

Cryopreservation

Cryopreservation is the only technique currently available to ensure the safe and cost-efficient long-term conservation of the germplasm of problem species. In this section, we briefly describe the various cryopreservation techniques available, summarize the achievements made and problems faced with vegetatively propagated and recalcitrant species and present the current utilization of cryopreservation for plant material.

Germplasm use

Biotechnological advances have offered new approaches to overcome challenges for effective utilization and enhancement of crop genetic resources. For instance, a number of techniques have been developed to overcome problems of sexual incompatibility that lead to hybrid sterility or lack of genetic recombination in wide crosses involving distant wild relatives and cultivated species.

Embryo rescue

In embryo rescue, an otherwise non-viable hybrid embryo is transferred to a culture medium where viable plants may be regenerated and backcrossed to the cultivated species to introduce the desired genetic trait. There are several examples of application of embryo rescue for a wide range of agronomically important species. Early examples of successful wide crosses using embryo rescue technique are: interspecific hybrids between *Lycopersicon esculentum* and *L. peruvianum* (Thomas and Pratt, 1981), and *Medicago sativa* and *M. rupestris* (McCoy, 1985). Hybrids have been obtained between *Arachis hypogaea* and the incompatible species *A. paraguariensis* and *A. appressipila,* both resistant to early leaf spot, using embryo rescue and tissue culture techniques (Rao et al., 2003). A combination of ovary culture and embryo rescue was used to develop fertile hybrid plants from the intergeneric cross between *Brassica napus* and *Sinapsis alba*, which has many desirable traits like resistance or tolerance to all major insect pests of brasicca crops, tolerance to high temperatures and drought besides being shatter resistant (Brown et al., 1997; Momotaz et al., 1998).

Embryo culture proved to be a useful tool to overcome postzygotic incompatibility in different *Helianthus* spp. And facilitated transfer of resistance to broomrape (*Orobanche cernua*) (Sunko et al., 1999). Recently, resistance to late blight from *Solanum pinnatisectum* was introgressed into *S. tuberosum* using embryo rescues and double pollination (Ramon and Hanneman, 2002).

Somatic hybridization

Protoplast fusion and somatic hybridization provide an alternative way for transfer of traits between distantly related species. It has been particularly useful in breeding programs to transfer beneficial characteristics from wild and weedy plants to the cultivated crop species, breaking the barrier for gene transfer. Protoplast surfaces bear strong negative charges, and intact protoplasts in suspension repel each other.

Hence fusion is accomplished by addition of calcium ions or polyethylene glycol (PEG) or using electric fields. Successful gene transfer via protoplasm fusion depends on the ability to regenerate a mature plant from the fusion product. There are several examples where pre-zygotic sexual incompatibilities are overcome using somatic protoplast fusion and plant regeneration from the heterokaryons formed by interspecies protoplast fusions. Thus, intertribal somatic hybrids were produced between *Brassica napus* and *Thlaspi perfoliatum* with high contents of nervonic acid (Fahleson et al., 1994).

Recently, protoplast fusion between *B. napus* and related wild species *Orychophragmus violaceus* enabled the transfer of genes for desirable fatty acid composition into *B. napus* (Hu et al., 2002). *Porteresia* is a halophytic species which can withstand total submergence in seawater and taxonomically related to rice (*Oryza sativa*). Pre-zygotic incompatibilities have resulted in the species being recalcitrant to sexual hybridization with *O. sativa*. Production of heterokaryons by the fusion of mesophyll protoplasts of *P. coarctata* and cell suspensions derived from protoplasts of *O. sativa* has been achieved and somatic hybrid plants have been produced after regeneration (Jelodar et al., 1999). Somatic hybrids were produced and late blight resistance was successfully transferred from *Solanum nigrum* into *S. tuberosum* (Horsman et al., 1997; Zimnoch-Guzowska et al., 2003).

CONCLUSION

In this paper, we have presented the new possibilities provided by biotechnologies for improving ex situ conservation of plant biodiversity in genebanks and botanic gardens. During recent years, dramatic progress has been made with the development of new conservation techniques for non-orthodox and vegetatively propagated species, especially in the area of cryopreservation, and the current ex situ conservation concepts should be modified accordingly to accommodate these technological advances. It is now well recognised that an appropriate conservation strategy for a particular plant genepool requires a holistic approach, combining the different ex situ and in situ conservation techniques available in a complementary manner. In situ and ex situ methods, including a range of techniques for the latter, are options available for the different genepool elements. Selection of the appropriate methods should be based on a range of criteria, including the biological nature of the species in question, practicality and feasibility of the particular methods chosen (which depend on the availability of the necessary infrastructures), as well as the cost-effectiveness and security afforded by their application. As already mentioned in this paper, the complementarity of genebank and botanic conservation should be fully recognised and capitalized upon to optimize plant biodiversity conservation. Considerations of complementarity with respect to the efficiency and cost-effectiveness of the various conservation methods chosen are also important. In many instances, the development of appropriate complementary conservation strategies will still require further research to define the criteria, refine the methods and test their application for a range of genepools and situations. In this context, it is important to stress that the new, efficient in vitro conservation techniques developed are not seen as replacements for conventional ex situ approaches. They offer genebank and botanic garden curators additional tools to allow them to improve the conservation of germplasm collections placed under their responsibility.

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