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Review

Plant genetic resources: Advancing conservation and use through biotechnology

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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.

Key words: Biotechnology, conservation, plant genetic resources.

INTRODUCTION

Plant genetic resources for food and agriculture are the basis of global food security. They comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species. Genetic diversity provides farmers and plant breeders with options to develop, through selection and breeding, new and more productive crops, that are resistant to virulent pests and diseases and adapted to changing environments. The world population is expected to reach eight billion by the year 2020 and food grain production will have to be doubled from the current level of about five billion tonnes per year. To meet the need for more food, it will be necessary to make better use of a broader range of the world's plant genetic diversity. Yet, genetic resources are disappearing at unprecedented rates. The reasons for this loss are many and include deforestation, developmental activities such as hydroelectric projects, road laying, urbanization and changes in agricultural practices, and finally modern agriculture and introduction of new and uniform varieties. More than 15 million hectares of tropical forest are lost each year. Genetically uniform modern varieties are replacing the highly diverse local cultivars and landraces in traditional agro-ecosystems. Over-grazing and changes in land-use pattern are taking heavy toll on diversity available in the wild species. Urbanization and changing life styles, globalization and market economies are also contributing indirectly to the loss of diversity, particularly of minor and neglected crops. Such reductions have serious implications for food security in the long term.

Global concern about loss of valuable genetic resources prompted international action. Programs for conservation of plant genetic resources for food and agriculture were thus initiated and genebanks established in many countries. The main objective was to collect and maintain the genetic diversity in order to ensure its continued availability to meet the needs of different users. The concept of germplasm conservation demands that collection methods initially capture maximum variation and subsequently, conservation and regeneration techniques minimize losses through time (Astley, 1992). To this effect, plant genetic resources (PGR) conservation activities comprise of collecting, conservation and management, identification of potentially valuable material by characterization, and evaluation for subsequent use. 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. I have attempted to review some of the recent progress in application of biotechnology to the advancement of plant genetic resources activities.

GERMPLASM COLLECTING

Collecting involves gathering samples of a species from populations in the field or natural habitats for conservation and subsequent use. The unit of collection may be botanic seeds or vegetative propagules, depending on the breeding system of the species. Collecting may be easy in species producing small botanic seeds in abundance. However, it becomes problematic when seeds are unavailable or non-viable due to: damage of plants by grazing or diseases; large and fleshy seeds that are difficult to transport; or where samples are not likely to remain viable during transportation due to remoteness of the collecting site from the genebank. Advances in biotechnology provide useful solutions for collecting such problem species (Withers, 1995). For example, in coconut (*Cocos nucifera*), where the major difficulty for standard seed collection is the large size of the seeds, *in vitro* techniques have been developed that allow collecting of the relatively small zygotic embryos in the field and transporting them back in sterile conditions to the laboratory to inoculate and germinate them on a culture medium (Assy-Bah et al., 1989; Ashburner et al., 1996). In cacao (*Theobroma cocoa*), where collecting germplasm in the field is limited by the rapid deterioration of samples during transit as the seeds do not withstand desiccation, Yidana et al. (1987) reported a simple *in vitro* method that involved collecting shoot nodal cuttings, followed by sterilization and inoculation of tissue into prepared culture vials containing semi-solid medium (see Withers and Engelmann, 1997). *In vitro* collecting methods were also developed for a range of other species including oil palm, forage grasses, banana, coffee, grape, *Prunus* and *Citrus* spp. (see Withers and Engelmann, 1997).

CONSERVATION

There are two approaches for conservation of plant genetic resources, namely *in situ* and *ex situ*. *In situ* conservation involves maintaining genetic resources in the natural habitats where they occur, whether as wild and uncultivated plant communities or crop cultivars in farmers' fields as components of the traditional agricultural systems. *Ex situ* conservation on the other hand, involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement or deterioration. Approaches to *ex situ* conservation include methods like seed storage, field genebanks and botanical gardens. DNA and pollen storage also contribute indirectly to *ex situ* conservation of PGR.

Among the various *ex situ* conservation methods, seed storage is the most convenient for long-term conservation of plant genetic resources. This involves desiccation of seeds to low moisture contents and storage at low temperatures. However, there are a large number of important tropical and sub-tropical tree species which produce recalcitrant seeds that quickly lose viability and do not survive desiccation, hence conventional seed storage strategies are not possible (Roberts, 1973). There are also a number of other important crop species that are sterile or do not easily produce seeds, or seed is highly heterozygous and clonal propagation is preferred to conserve elite genotypes. Examples of crop species in this category are banana, sweet potato, sugarcane, cassava, yam, potato, and taro. These species are usually conserved in field genebanks. Although field genebanks provide easy access to conserved material for use, they run the risk of destruction by natural calamities, pests and diseases. For this reason, safety duplicates of the living collections are established using alternate strategies of conservation and it is in this area that biotechnology contributed significantly by providing complementary *in vitro* conservation options through tissue culture techniques. *In vitro* conservation also offers other distinct advantages. For example, the material can be maintained in a pathogen-tested state, thereby facilitating safer distribution. Further, the cultures are not subjected to environmental disturbances (Withers and Engelmann, 1997).

Several *in vitro* techniques have been developed for storage of vegetatively propagated and recalcitrant seed producing species. In general, they fall under two categories: (i) slow growth procedures, where germplasm accessions are kept as sterile plant tissues or plantlets on nutrient gels; and (ii) cryopreservation where plant material is stored in liquid nitrogen. Slow growth procedures provide short- and medium-term storage options, while cryopreservation enables long-term storage of the plant material. For more details on the use of biotechnology tools such as *in vitro* and cryopreservation methods, see Engelmann and Engels (2002).

Slow growth

Slow growth procedures allow clonal plant material to be held for 1-15 years under tissue culture conditions with periodic sub-culturing, depending on species. There are several methods by which slow growth can be maintained.

In most cases, a low temperature often in combination with low light intensity or even darkness is used to limit growth. Temperatures in the range of 0-5ºC are employed with cold tolerant species, but for tropical species which are generally sensitive to cold, temperatures between 15º and 20ºC are used. It is also possible to limit growth by modifying the culture medium, mainly by reducing the sugar and/or mineral elements concentration and reduction of oxygen level available to cultures by covering explants with a layer of liquid medium or mineral oil (Withers and Engelmann, 1997).

Regeneration and successful propagation of genetically stable seedlings from cultures are prerequisites for any in vitro conservation effort. Protocols for clonal multiplication are well established for several species (see Ashmore, 1997). Generally, organized cultures such as shoots are used for slow growth storage since undifferentiated tissues such as callus are more vulnerable to somaclonal variation. Although slow growth procedures have been developed for a wide range of species, they are routinely used for conservation of genetic resources of only a few species including *Musa* spp., potato, sweet potato, cassava, yam, *Allium* spp. and temperate tree species. About 37,600 accessions are reportedly conserved by *in vitro* techniques in genebanks, worldwide (FAO, 1996).

Cryopreservation

Cryopreservation involves storage of plant material at ultra-low temperatures in liquid nitrogen (-196°C). At this temperature, cell division and metabolic activities remain suspended and the material can be stored without changes for long periods of time. Cryopreservation is the only available method for long-term conservation of vegetatively propagated plant germplasm. The choice of material includes cells, protoplasts, shoot apices, somatic embryos, seed or excised zygotic embryos. Cryopreservation requires limited space, protects material from contamination, involves very little maintenance and is considered to be a cost-effective option.

The techniques for cryopreservation currently in use are quite varied and include the older classical techniques based on freeze-induced dehydration of cells as well as newer techniques based on vitrification (Engelmann, 2000). In classical techniques, tissues are cooled slowly at a controlled rate (usually 0.1-4°C/min) down to about –40ºC, followed by rapid immersion of samples in liquid nitrogen. Slow freezing is carried out using a programmable freezing apparatus. Cryoprotectants are added to the freezing mixtures to maintain membrane integrity and increase osmotic potential of the external medium. Classical cryopreservation procedures have been successfully applied to undifferentiated culture systems such as cell suspensions and calluses (Kartha and Engelmann, 1994). However, in case of differentiated structures, they have been employed for storage of apices or embryonic axes of only cold-tolerant species (Reed and Chang, 1997), and their utilization for tropical species has been limited (Escobar et al., 1997). Vitrification-based procedures involve removal of most or all freezable water by physical or osmotic dehydration of explants, followed by ultra-rapid freezing which results in vitrification of intracellular solutes, i.e. formation of an amorphous glassy structure without occurrence of ice crystals which are detrimental to cellular structural integrity. These techniques are more appropriate for complex organs like embryos and shoot apices; they are also less complex and do not require a programmable freezer, hence are suited for use in any laboratory with basic facilities for tissue culture. Engelmann (2000) described seven vitrification-based procedures in use for cryopreservation: (1) encapsulation-dehydration, (2) vitrification, (3) encapsulation-vitrification, (4) desiccation, (5) pregrowth, (6) pregrowth-desiccation, and (7) droplet freezing. With the advent of these new cryogenic procedures, especially vitrification, encapsulation-vitrification and encapsulation-dehydration, the number of species cryopreserved has increased significantly in recent years.

In general, cryopreservation is well established for vegetatively propagated species. However, it is much less advanced for recalcitrant seed species due to some of their characteristics, including their very high sensitivity to desiccation, structural complexity and heterogeneity in terms of developmental stage and water content at maturity. The new cryopreservation techniques have been successfully applied for more than 80 species (see Engelmann, 1997; Reinhoud, 2000; Takagi, 2000) and they are under development or vigorous testing for several other species. However, examples of their routine use for long-term conservation are still limited only to oil palm and potato (see Engelmann, 1997). As research carried out by various teams worldwide is progressively improving our understanding of mechanisms involved in cryopreservation, it is expected that the utilization of cryopreservation in genetic resources conservation will increase steadily in the coming years.

CHARACTERIZATION OF DIVERSITY

The ability to identify genetic variation is indispensable to effective management and use of genetic resources. Traditionally, diversity is assessed by measuring variation in phenotypic traits such as flower colour, growth habit or quantitative agronomic traits like yield potential, stress tolerance, etc., which are of direct interest to users. This approach has certain limitations: genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence. In the 1960s, biochemical methods based on seed protein and enzyme

electrophoresis were introduced, which proved particularly useful in analysis of genetic diversity as they reveal differences between seed storage proteins or enzymes encoded by different alleles at one (allozymes) or more gene loci (isozymes). Use of biochemical methods eliminates the environmental influence; however, their usefulness is limited due to their inability to detect low levels of variation. DNA-based techniques introduced over the past two decades have potential to identify polymorphisms represented by differences in DNA sequences. These methods are being used as complementary strategies to traditional approaches for assessment of genetic diversity, the major advantage being that they analyse the variation at the DNA level itself, excluding all environmental influences. The analysis can be performed at any growth stage using any plant part and it requires only small amounts of material. Following the advances in molecular biology in the last decade, a variety of different methods have been developed for analysis of genetic diversity. These methods differ with respect to technical requirements, level of polymorphism detected, reproducibility and cost (see Karp et al., 1997; Karp, 2002).

Molecular methods used for detecting DNA sequence variation are generally based on the use of restriction enzymes that recognize and cut specific short sequences of DNA (e.g., Restriction Fragment Length Polymorphism, RFLP) or polymerase chain reaction (PCR), which involves amplification of target DNA sequences using short oligonucleotide primers. PCR-based techniques such as Random Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs, microsatellites) have proved especially important in diversity studies. Newer and more powerful molecular techniques that detect variation at specific gene loci, which can be automated for high throughput of samples, are becoming available (e.g. Sicard et al., 1999), permitting precise and versatile analyses of genetic variation. Nevertheless, it is difficult to compare the different techniques and determine which one is best and for what purpose because each method has its advantages and disadvantages. The appropriateness of individual marker systems also varies depending on the objective of study and the properties of the species (Karp et al., 1997).

Molecular methods are increasingly playing an important role in conservation and use of plant genetic resources. Recently, Hodgkin et al. (2001) reviewed the techniques available and the ways in which they are used for analysis of diversity to support PGR activities. Specific areas in which molecular marker techniques have been used are: developing sampling strategies and identification of gaps in the collections to plan for future collecting and acquisition, and managing conserved germplasm – including identification of redundancies, development of core collections, fingerprinting, identification of genetic contamination and quantification of genetic drifts/shifts.

Sampling strategies

Molecular markers have been applied to study genetic diversity from natural populations and formulate efficient sampling strategies to capture maximum variation for genetic resources conservation. For example, the substantially higher level of RFLP variation observed in self-incompatible, as compared with self-compatible species of *Lycopersicon* was used to recommend predominant sampling of self-incompatible species for germplasm acquisition (Miller and Tanksley, 1990). Studies of distribution of genetic diversity using AFLP markers in Sri Lankan coconut populations showed that emphasis should be placed on collecting relatively large numbers of plants from few populations since most of the diversity is within populations rather than between populations (Perera et al., 1998). Genetic variation within and between natural populations of *Digitalis obscura* was quantified using RAPDs and the results were used for optimizing sampling strategies for conservation of genetic resources of the species (Nebauer et al., 1999). Recommendations to focus on the sampling of marginal pawpaw (*Asimina triloba*) populations in future collection missions were derived from the genetic structure across natural distribution areas, established by RAPD analysis (Huang et al., 2000).

Managing genetic resources

Molecular techniques proved useful in a number of ways to improve the conservation and management of PGR. In particular, genetic diversity data provides information on gaps in terms of coverage in gene pools as well as redundancies, i.e., material with similar characteristics that wastes resources through increased cost of management. For example, RAPD analysis in *Brassica oleracea* revealed that 14 phenotypically uniform accessions could be reduced to 4 groups with minimal loss of genetic variation (Phippen et al., 1997). Intra-accession variation was determined by AFLP markers in *ex situ* conserved barley, and results were used to evaluate the efficiency of splitting heterogeneous accessions into distinct lines in order to avoid the negative effects of selection and genetic drift during regeneration (van Treuren and van Hintum, 2001). Recent examples of use of molecular markers to identify redundancies in collections include perennial kales (Zeven et al., 1998), wheat (Cao et al., 1998), grapevine (Cervera et al., 1998), sorghum (Dean et al., 1999), cassava (Chavarriage et al., 1999), flax (van Treuren et al., 2001), and barley (Lund et al., 2003).

Molecular markers have been used to identify groups

from which core collection accessions can be selected or to monitor the effectiveness of one or the other strategy in capturing genetic diversity found in the whole collection. For example, molecular markers were used to assist the assembly of a core collection for cultivated potato species *Solanum phureja* (Ghislain et al., 1999). Using RAPD markers, Skroch et al. (1998) found no significant differences in genetic diversity between core collection and a chosen set of accessions, randomly chosen from the whole collection of common bean germplasm, indicating that the method used to develop the core collection had not been very efficient in capturing diversity. Grenier et al. (2000) evaluated different sampling methods to constitute a core collection of sorghum landraces for the extent of genetic diversity captured based on microsatellites analysis.

Molecular markers have been employed for fingerprinting, verification of accession identity and genetic contamination. For example, microsatellites were used to distinguish different cultivars of grapevine (Thomas et al., 1994), and to compare landraces and develop unique DNA profiles of soybean genotypes (Rongwen et al., 1995). Hongtrakul et al. (1997) and Fang et al. (1997) used molecular markers as tools to fingerprint sunflower and trifoliate orange germplasm accessions, respectively. RAPDs were used to identify dwarf off-types arising from micropropagation of banana cultivars (Damasco et al., 1996) and incorrectly labelled accessions in a barley germplasm (Poulsen et al., 1996). Del Rio et al. (1997), Wu et al. (1998) and Börner et al. (2000) using RAPDs studied the changes in genetic diversity following regeneration of potato, rapeseed and wheat accessions, respectively.

Variation within species has also been studied to explore geographic or ecological patterns of distribution of diversity in many different crops and their wild relatives that include wild bean (*Phaseolus vulgaris*) (Tohme et al., 1996), banana (Pillay et al., 2001), mango (Kashkush et al., 2001), bambara groundnut (Massawe et al., 2002), vetch (Potokina et al., 2002), *Cicer* spp. (Iruela et al., 2002), sorghum (Nkongolo and Nsapato, 2003), sweet potato (Gichuki, 2003), tea (Balasaravanan et al., 2003) and chicory (Van Cutsem et al., 2003). In *Lycopersicon*, microsatellites analysis revealed high level of polymorphism within and among species, which was highly correlated with the respective mating systems, cross -pollinating species having a significantly higher gene diversity compared to self-pollinating species (Alvarez et al., 2001). AFLP marker analysis revealed limited genetic diversity within and between sites, when Fajardo et al. (2002) studied genetic diversity in sweet potato accessions collected from four provinces of Papua New Guinea, a secondary centre of diversity. Analysis of genetic diversity in 61 accessions of *Coffea arabica* and two other diploid species using AFLP markers revealed highest level of genetic diversity within the cultivar Catimor (Steiger et al., 2002). Ude et al. (2003) used AFLP and RADP markers to detect polymorphism and assess genetic relationships in a set of 25 plantains from Western and Central Africa. A small group of genetically distinct cultivars from Cameroon was separated from the bulk of other plantains, suggesting that Cameroon may harbour accessions with useful or rare genes for widening the genetic base of breeding populations.

Molecular markers are being increasingly used to resolve problems of taxonomy and phylogenetic relationships, as a good knowledge of genomic homologies helps in devising suitable breeding strategies for appropriate conservation as well as transfer of genes from one species to another. Ramanatha Rao and Riley (1994) reviewed some of the earlier studies in this area. Some recent examples are: Rossetto et al. (2002) examined the taxonomic relationship between *Vitis vinifera* and *Cayratia saponaria* using microsatellites and found sufficient inter-specific variation to distinguish the two very closely related species. Xu et al. (2002) surveyed the variation in chloroplast DNA simple sequence repeats (cpSSR) in wild and cultivated soybean accessions collected from various Asian countries. Analyses indicated a considerably higher genetic diversity in the wild soybean and the results suggested that cultivated soybeans originated independently in different regions from different wild gene pools and /or hybrid swarm between cultivated and wild forms. RAPDs were used to assay genetic variation and establish genetic relationships among the wild species of *Arachis*. The relationships were similar to those previously reported based on morphological, cytological and crossability data (Santos et al., 2003). AFLPs were used to validate taxonomic classification, to investigate the extent of redundancy and distribution of genetic diversity across the collection area in wild potato species of the series Acaulia. Cluster analysis of the AFLP data grouped them according to the species and subspecies, and identified some 16 misclassifications, including four that did not seem to belong to the series (McGregor et al., 2002). Isshiki et al. (2003) used RFLP analysis of mitochondrial DNA in *Solanum melongena* and six related *Solanum* species, for assessing phylogenetic relationships. Tang and Knapp (2003) performed phylogenetic analysis on domesticated and wild germplasm accessions of sunflower using microsatellite loci, which uncovered the possibility of multiple domestication origin in sunflower.

In situ **conservation**

Diversity studies using molecular markers have also assisted in developing *in situ* conservation strategies. For example, Chalmers et al., (1994) studied genetic diversity among eight mahogany species using RAPD analysis and the results were discussed in relation to development of genetic conservation and improvement strategies.

RAPDs were employed to assist a recovery programme for threatened perennial *Grevillea scapigers* in south western Australia (Rossetto et al., 1995) and to guide domestication programmes and conservation approaches for bush mango species from Central and West Africa (Lowe et al., 2000). Recently, Pavek et al. (2003) assessed genetic variation in populations of rock grape (*Vitis rupestris*) using SSR markers in combination with morphological data and identified seven sites for *in sit*u conservation in the USA.

The advent of molecular genetic markers made it easy to discriminate between wanted and unwanted agronomic genes in segregating populations. If linkages are established between a heritable agronomic trait and a genetic marker, markers can be used to identify the location of genes. Such linkages allow direct selection for the trait using marker assisted selection in a backcrossing programme. Molecular markers which densely cover an entire crop genome can be applied to develop a molecular map for a crop, which could be used to determine linkage between a specific molecular marker and a strongly heritable trait. This holds great promise for breeding programmes as many traits are difficult to select for directly from breeding populations.

GERMPLASM EXCHANGE

The ability to store and exchange healthy germplasm is fundamental to effective conservation and use of plant genetic resources. Infected propagules are known to deteriorate quickly in sub-optimal storage conditions, therefore it is important to store only healthy samples in the genebanks. Further, because planting material is an international commodity used for exchange of germplasm, infected seeds inadvertently serve as means of introducing plant pathogens into new areas. For the above reasons, seed health tests and phytosanitary regulations are generally incorporated as part of the genebank activities. Prior to the 1970s, seed health tests were primarily directed towards fungi and relied on incubation or grow -out tests for detection of pathogens on the seed. Such tests are labour intensive, time consuming and in some cases seed require high levels of infection before pathogens can be detected. The need to use representative sample sizes in the traditional grow-out methods to detect pathogen contamination also poses a problem when only limited amount of seed is available for genetic conservation. Also, pathogens that are symptomless such as viruses and viroids pose great risk in exchange of germplasm as they cannot be detected by traditional methods of bioassay. The problems are more severe for crops propagated by vegetative means, as viruses and viroids are transmitted very efficiently through vegetative propagules.

The demand to store and exchange seeds of high quality and for greater sensitivity in detecting seed-borne pathogens including viruses and bacteria and short-turnaround times for seed testing have led many genebanks and seed health testing laboratories develop or adopt new technologies. Barker and Torrance (1997) recently reviewed the increasing role and future potential of biotechnology in detection of pathogens from germplasm collections. In lettuce, for example, the method for detection of lettuce mosaic virus (LMV) changed since 1983 and a serological test - enzyme-linked immunosorbent assay (ELISA) using specific LMV antiserum was developed replacing the standard seed assays which require a grow-out and evaluation of 30,000 lettuce seedlings (Falk and Purciful, 1983). ELISA is not only more efficient, but it has proved to be sensitive in finding low levels of seedborne LMV that could devastate lettuce production. Immunofluoresence is an improved technology that is routinely used for screening seed samples for bacterial pathogens.

The PCR technique used to amplify small amounts of DNA, also improves the efficiency of seed health tests, besides sensitivity and/or specificity. For example, in watermelon, PCR has been developed for detecting bacterial fruit blotch pathogen as an alternative to grow-out tests requiring 10,000 seedlings (Minsavage et al., 1995). DNA primers have been developed for several important seedborne pathogens, and PCR techniques are in routine use for the detection of blackleg of crucifers (Taylor, 1993), halo blight of beans (Schaad et al., 1995) and bacterial canker (CMM) of tomato (Santos and Cruz, 1997). Diagnostic tools that use oligonucleotide-Probe (DOP) hybridization are now available to detect coconut cadang-cadang viroid (Hodgson and Randles, 1997).

Detection of pathogens has to be followed by elimination for safe exchange or conservation of the material. Apical meristem culture has been used to successfully eliminate many viruses from a variety of plant species. Meristem culture is usually combined with heat treatment for better results. Ng (1994) described tissue culture techniques for both disease elimination and micropropagation in cassava. Postman (1994) reported successful elimination of viruses from 400 pear cultivars conserved at USDA-ARS National Clonal Repository. Thermotherapy and meristem-tip culture were used for elimination of viruses from cassava collections in the *in vitro* genebank at the International Center for Tropical Agriculture (CIAT) (IPGRI/CIAT 1994). Other reports of successful elimination of viruses from important crop species include cassava mosaic virus from cassava (Kartha and Gamborg, 1975), exocortis viroid from *Citrus* species (Greno et al., 1990), potato virus S from potato (Brown et al., 1988) and sweet potato yellow dwarf virus from sweet potato plants (Green and Lo, 1989).

Exchange of germplasm as *in vitro* cultures offers considerable advantages like reduced volume and weight as well as the improved health status of the cultures. Crop species for which *in vitro* germplasm exchange is a routine include *Musa* spp., potato, yam and cassava.

Cultures are generally transported as shoot cultures on standard media, or as microtubers in case of tuber crops like potato, sweet potato and yam (Espinoza et al., 1992; Ng, 1994). In banana, Rao et al. (1993) reported the use of shoot tips encapsulated in alginate beads and in yam and potato, Hasan and Takagi (1995) described the use of encapsulated nodal segments as an alternative form for distribution of germplasm. These techniques, besides reducing the weight and volume of the material to be distributed, retain the sterile conditions and simplify the process of regeneration upon receipt of the 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 (see 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 post-zygotic 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, 2003).

CONCLUSION

As reviewed above, biotechnology has made significant contributions to improved conservation and use of plant genetic resources. The rapid progress made in *in vitro* culture technology has helped in improving the conservation of genetic resources especially of problem species. Some of the most important contributions have been in the areas of *in vitro* collecting, slow growth and cryopreservation. Slow-growth techniques are in a more advanced state of development than cryopreservation techniques, which still require improvement before they can be used on a routine basis in a number of species. Tissue culture technology has also assisted in production of disease-free material for exchange, and novel genetic combinations have been achieved through embryo rescue and somatic hybridisation. By facilitating better understanding of diversity, both in extent and structure,

molecular marker techniques are proving extremely useful in identification of redundancies in collections, in testing accession stability and integrity, and in supporting the development of effective management strategies both for *ex situ* and *in situ* conservation. Molecular genetic studies are also being increasingly used to support improved use of plant genetic resources. The sequence data that are becoming available for increasing numbers of genes as a result of the rapid advances in DNA technology have stimulated the development of novel molecular technologies which allow the screening of germplasm for functional diversity and identify variation at an early developmental stage without the need for performing the time-consuming evaluation tests, such as pathogen resistance screening. Despite the progress made, there remain many unresolved questions, the most important being that of determining the most appropriate molecular markers for the required understanding of the patterns of diversity in specific studies. While there is a pressing need to ensure that available technologies are made accesible to a wider range of users through improved training and other capacity building initiatives, the existing technologies are also expensive and given that most of the crop diversity is to be found in developing countries, the issue of resources assumes importance (Hodgkin et al., 2001). Hence, there is real need to maximize synergy through appropriate collaboration between various national, sub-regional and international levels, including sharing burdens and responsibilities, in order to use these techniques for effective conservation and use of plant genetic resources.

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