Journal of Biotechnology, 17 (1991) 247–256

ADONIS 0168165691000657

BIOTEC 00580

Biotechnology and methods of conservation of plant genetic resources

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(Received 2 August 1990; accepted 17 August 1990)

Genetic diversity; Germplasm; Conservation; Genebank; Cryopreservation

Introduction

The loss of biological diversity is of major environmental concern at the present time. Whether this relates to the destruction of the tropical rain forests, for instance, or the threat which faces natural ecosystems as climates change due to global warming (Jackson et al., 1990), it is important that in order to survive and continue to evolve, plant and animal species must contain an ample reservoir of genetic diversity.

One aspect of this process is the continuing loss of genetic diversity in the crops upon which world agriculture is based. Scientists throughout the world are rightly engaged in developing better and higher yielding cultivars of crop plants to be used on increasingly larger scales. But this involves the replacement of the generally variable, lower yielding, locally adapted strains grown in traditional farming systems, by the products of modern agriculture. Nowadays every major crop has a relatively narrow genetic base, and so under such situations, diversity in farming systems is replaced by genetic uniformity. However, in order to be able to respond to the various stresses that threaten modern agriculture, plant breeders are dependent upon the availability of a pool of diverse genetic material.

The significance of genetic uniformity can be highlighted by several crop examples, one of historical importance and two others of more recent times. The history of the potato in Europe illustrates the necessity for utilizing genetic resources to

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broaden the genetic base of crop plants. The narrow genetic base of the potato crop was recognised during the last century following the devastating epidemics of late blight disease, caused by *Phytophthora infestans*, in the 1840s in Ireland. Massive destruction of the corn crop came about in the United States in 1970 with the southern corn leaf blight epidemic (caused by the fungus *Helminthosporium maydis*). Modern plant breeding had led to the use of a cytoplasmic gene which conferred susceptibility to a particular race of this fungal pathogen. A more recent, but less publicised case of genetic vulnerability was caused not by plant disease, but by cold weather. By 1972 in the Soviet Union the wheat variety 'Bezostaja' was grown on almost 15 million hectares. It had been moved beyond its original area of cultivation far into the Ukraine during a period of relatively mild winters. Then in 1972 a very severe winter occurred, causing losses of millions of tons of winter wheat (Fischbeck, 1981). In relation to this example, we can ask whether global warming will lead to similar crop losses in the future (Jackson and Ford-Lloyd, 1990).

Plant genetic resources (germplasm) is a term used to describe the total genetic diversity of cultivated species and their wild relatives, much of which may be of value to breeders. Although commercial and obsolete varieties, breeders' lines and induced or natural mutations may be included under this term, it is much more usual to describe plant genetic resources as landraces or primitive forms, weed races which are closely related to the cultivated species, and related wild species. Landraces are populations of crops, often collected from remote areas, where the new, highly bred cultivars have not been introduced. They are highly diverse genetically, and have often been grown as mixtures of species, as well as diverse populations of one species. Such materials are closely allied genetically to modern varieties and are extremely important genetic resources, and as such have received top priority for conservation.

Conservation methods

The relevance of biotechnology to the conservation of plant genetic resources has to be viewed in terms of conservation strategies. The strategy employed for the conservation of germplasm depends very much on the nature of the material. There are two principal strategies, termed in situ conservation and ex situ conservation. In situ conservation is applied mainly to wild species' relatives of crop plants, to forest and pasture species. It is often recommended that such species should be preserved, maintaining the genetic integrity of their natural state, as communities in stable environments. Most nature conservation programmes are aimed at the level of ecosystems. Genetic conservation goes further in recognising the need for a wide genetic base, and as with nature conservation, aspects of population biology such as adaptive radiation, maintenance of variation in populations, and the long-term stability of population numbers are important features (Ford-Lloyd and Jackson, 1986).

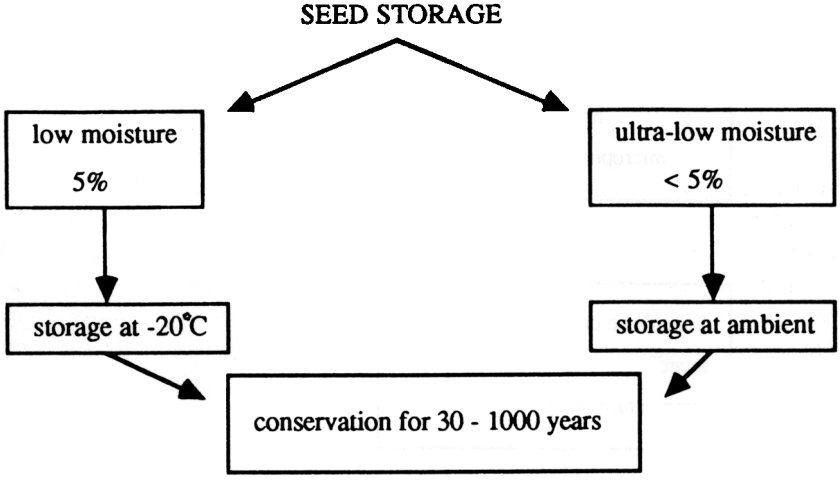
The situation of the primitive cultivars, or landraces is very different from that of wild species. These categories evolved in association with humans in the agricultural

environment, or agro-ecosystem. The application of in situ conservation of landraces is not a feasible option, and because of the rapid loss of genetic variation through cultivar replacement, ex situ conservation in one form or another is the most practical and safe approach for such material.

In the broad sense, ex situ conservation includes the use of botanic gardens and arboreta, and more importantly, gene banks. Genetic conservation in gene banks is static conservation (Guldager, 1975), which is aimed at retaining as far as possible the structure of the original population. The aim is to prevent loss of genetic information. Static conservation mainly takes the form of the storage of seeds or vegetative material in gene banks. Conservation carried out in this way by reducing the life processes to a low level is both the safest and the cheapest method.

Conservation in seed genebanks

For the majority of crop plants and many of their wild relatives, seeds can be maintained with retention of viability for long periods of time under conditions of decreased moisture content and low temperature (Fig. 1). Under storage conditions of $-20°C$ and 5% moisture content, it is predicted that seeds of barley will retain adequate viability for over 70 years, rice for over 300 and pea for over 1000 years (Roberts, 1973; Roos, 1989). Genebanks with large controlled environment rooms running under these conditions can be found in many parts of the world. These hold



Limitations : inappropriate for vegetatively and clonally propagated crops

inappropriate for recalcitrant species

Fig. 1. Strategy for germplasm conservation: seeds.

large numbers of seed samples. However, small collections of germplasm can be stored equally well for very low cost in domestic deep-freezers.

Results of well-established research have already been translated into practical handbooks describing how best to store seed in genebanks (Ellis et al., 1985a,b). Current views indicate that recommendations for storing some seeds may need to change in the future. Seed storage longevity may be increased dramatically by storing seeds of some crops at an ultra-low moisture content of 2–3%. For *Brassica napus* seeds stored at 3% moisture instead of 5%, the half-viability is increased as much as 12 times (Ellis et al., 1989). Research has shown that the critical moisture content varies substantially among the species tested, and ranges from 2% to 6%, but nevertheless represents a cost-effective means of storing seeds and hence genetic resources, particularly in the tropics. This is demonstrated by the fact that a reduction from 5% to 2% in seed storage moisture content of rape seeds provides approximately the same increase in longevity as a reduction in storage temperature from $+20\,^{\circ}C$ to $-10\,^{\circ}C$.

In vitro techniques

There are genetic resources for which seed storage is not appropriate or even possible and therefore where biotechnology may play an expanding role in their future. Those species which are normally vegetatively propagated, or which do not produce viable seed, or those where the seeds produced are very short-lived (termed

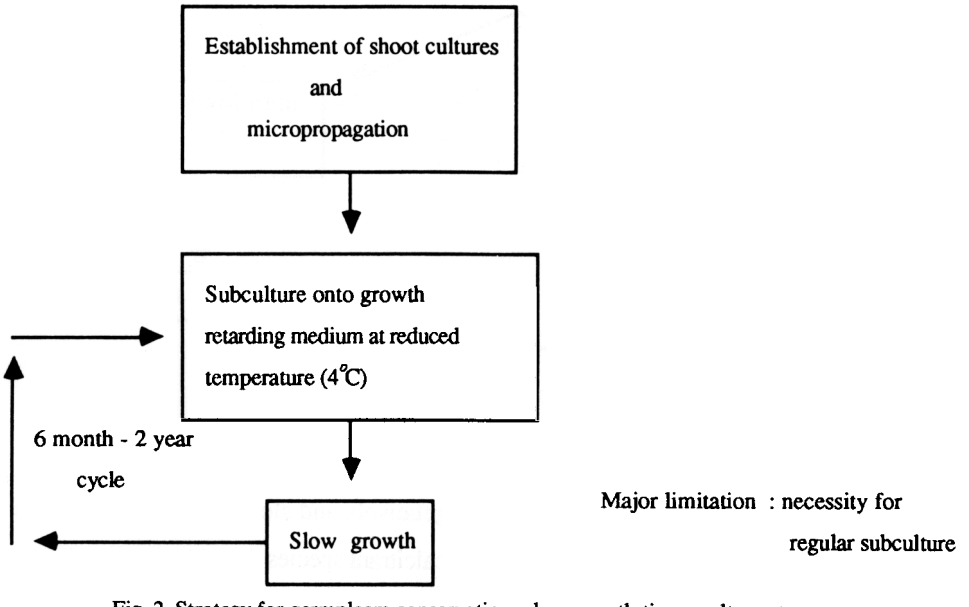


Fig. 2. Strategy for germplasm conservation: slow growth tissue culture storage.

recalcitrant) and are killed by exposure to low temperature and moisture, have to be conserved by other means. Many species of fruit, and some large-seeded tree species have seeds which are relatively short-lived and last no more than a few weeks or months. This includes such economically important species as cocoa, rubber, tea, most tropical fruits and many timber species. Vegetatively propagated crops such as potato, cassava, yam, sweet potato, sugar cane and temperate fruit trees present special problems for conservation, not because their seeds cannot be stored conventionally, but because it is not convenient to propagate them commercially from seed due to high levels of genetic heterozygosity, and because breeders and horticulturists commonly require uniform clones (Ford-Lloyd and Jackson, 1986). For these reasons much interest has been focussed on the application of tissue culture or in vitro techniques to plant genetic resource conservation. It is now possible to store plants in vitro for short periods of time, or longer if sub-culturing is carried out after certain intervals (Fig. 2). Such slow growth storage has proved extremely successful for shoot cultures of potato, cassava, fruit crops such as banana, apple, pear, strawberry and many other horticultural species (Withers, 1987). Strawberry has been stored for 6 years with occasional addition of water to the culture medium (Aitken-Christie and Singh, 1987), and garlic shoots can be stored for short periods involving sub-culturing every 18 to 24 months (El-Gizawy and Ford-Lloyd, 1987). Another exciting application is in vitro collection of germplasm in the field. Successful protocols have been reported for cocoa (Withers, 1987) and coconut (Assy-Bah et al., 1987).

Ultra-low temperature storage and genetic stability

In vitro technology also opens up the possibility of ultra-low temperature storage of vegetative material, or cryopreservation at temperatures as low as $-196\,^{\circ}C$. By this means germplasm could be stored indefinitely (Fig. 3). Cell suspensions and callus cultures can now be cryopreserved quite routinely in liquid nitrogen (Withers, 1985), and high survival (50–80%) can be achieved, along with stability of desirable traits (Watanabe et al., 1985). However, there exist doubts about genetic stability of such disorganised cultures in relation to the possible introduction of high levels of somaclonal variation amongst regenerants. It may well be that germplasm conservation will not be seriously affected, providing that regenerant plants from previously cryopreserved callus show only a small proportion of somaclonal variation. Biotechnological tools, including analysis of restriction fragment length polymorphism (RFLP) could be used to identify those regenerants which are variant genetically from the parental stock (Bernatzky and Tanksley, 1989).

Shoot cultures and meristems have proved to be much more difficult to freeze effectively (Withers, 1988) but are supposed to represent a higher level of genetic stability. Most success has been with dormant buds of *Prunus* derived from trees in vivo rather than in vitro (IBPGR, 1989). Despite the fact that mature cassava seeds as well as zygotic embryos are resistant to freezing and show little loss of viability on exposure to, and recovery from liquid nitrogen treatment, somatic embryos do

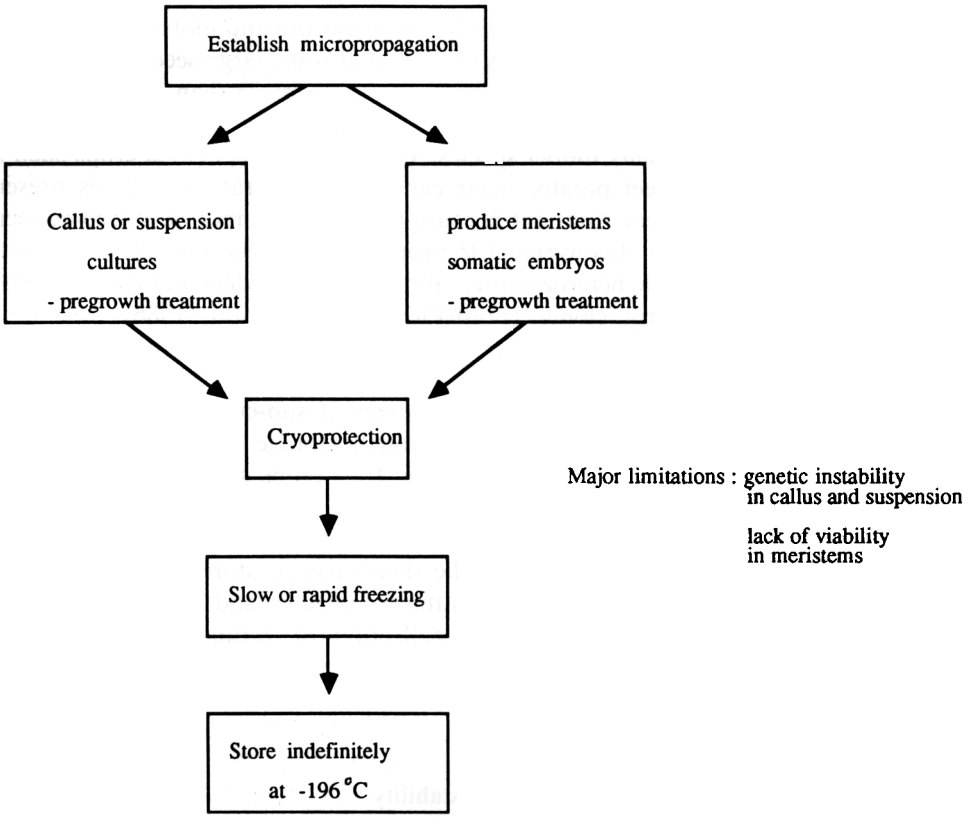


Fig. 3. Strategy for germplasm conservation: cryopreservation.

not survive. Nevertheless there is much interest being shown in the potential use of somatic embryos for genetic conservation. It has been possible to freeze-dry somatic embryos of carrot for some time (Withers, 1979). They can be recovered at a high frequency, and develop non-adventitiously, a fact which is recognised as being important for maintaining the genetic integrity of the material, and for avoiding somaclonal variation. Somatic embryos of oil palm are also remarkably resistant to different rates and methods of freezing (Engelmann et al., 1985). It may be that somatic embryos are generally more amenable to freezing because of their physiology and morphology. With several recent demonstrations that somatic embryos of different species can be desiccated or encapsulated to form 'artificial seeds' for commercial purposes, it is likely that for certain genetic resources, these novel man-made propagules may be important units of conservation in the future (Redenbaugh et al., 1986; Withers, 1988).

One caution in relation to any strategy for conservation involving the tissue culture of organs or cells needs to be heeded. It is now quite clear that much plant material exhibits genotypic variation for tissue culture ability. This is particularly the case for disorganised culture growth followed by regeneration from callus. Such

a factor would apply significant selection to germplasm which could be conserved (Coleman et al., 1990).

The problem of plant viruses and plant quarantine

Diseases of all types are a particular problem in vegetatively-propagated crop species, and may lead to restrictions in the free exchange and distribution of germplasm. It is important that in vitro cultures are free from diseases, and whilst fungal and bacterial pathogens can be eliminated quite effectively prior to culture in vitro, virus diseases present a special challenge for in vitro conservation.

It is illustrative to describe the situation with one of the world's major crops, the potato. This crop originated in the Andes of South America, where many virus diseases, unknown elsewhere in the world, are common. The International Potato Center (CIP), one of a group of internationally sponsored agricultural research centres, maintains the World Potato Collection, where many clones are conserved in vitro. Export of germplasm of seed and tuber lines in vitro is allowed only from virus-free materials. Virus elimination can be achieved by heat therapy, but it is now more common for virus elimination to be achieved in vitro using anti-viral compounds included in the culture medium. By sequentially subculturing meristems or nodal cuttings on a medium containing such substances, virus elimination has been achieved. Particular success has been reported with the synthetic riboside, ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), also known as Virazole. Several viruses of potatoes, including PVX, PVY, PVM and PVS, have been eliminated in this way (Cassells and Long, 1982; Klein and Livingston, 1982; Wambugu et al., 1985; Bittner et al., 1989).

Clearly such in vitro virus elimination techniques are very important both for the conservation of germplasm, but also in terms of its distribution and subsequent utilization in potato breeding. The chemotherapeutic methods may not yet have a wide application, but do represent a major advance in the conservation of genetic resources.

DNA libraries

The feasibility of storing the total genomic information of germplasm by way of DNA libraries has already been discussed in the literature (Peacock, 1989). Extraction of DNA followed by partial digestion with a restriction enzyme results in segments of DNA which can be stored easily and indefinitely even at room temperature (Fig. 4). Peacock indeed states that the genomes of all the plant species in the world could be stored in one small room by this method. There are of course major objections to this scenario. One of these is that current technology will only allow for the recovery of single genes, and not whole genomes, genome segments or even gene complexes: it is difficult to imagine now how it could be hoped to manipulate quantitative trait loci (QTLs). Also there is still the problem of being

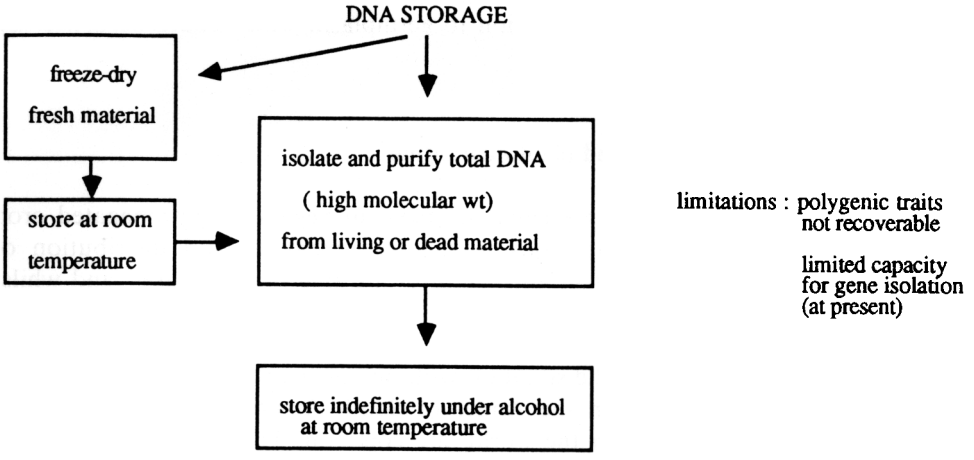


Fig. 4. Strategy for germplasm conservation: DNA storage.

able to identify many single genes of interest, in order for them to be subsequently cloned and transferred by existing genetic engineering techniques. The magnitude of the problem is also overlooked, in the sense that it is not a 'representative' genome of each species which needs to be stored in the form of DNA, but to be of any use as a genetic resource, DNA from many genotypes within a population, and of many populations within a species would need to be stored.

However, a positive case can be made that this latter problem of magnitude also applies to vegetatively propagated material currently stored in field genebanks or in vitro genebanks. The problem of adequate genetic representation is just the same. So, looking to the future and inevitable technological advances, it is worth considering the establishment of DNA libraries for certain categories of plant germplasm, at least alongside more conventionally stored germplasm. This would undoubtedly serve to make more readily available genetic resources to scientists working with genes at the molecular level.

The final conclusion to be reached when considering the current status of molecular biology in relation to genetic resources is that perhaps inviable seed lots should never be discarded from genetic resource collections, as DNA from such material, along with herbarium specimens may hold genes which will be utilizable in the future. It is already possible to isolate DNA in a form which is easily analysed, from small herbarium samples (Rogers and Bendich, 1985). In principle, there is no reason why genes from apparently extinct taxa could not be identified, cloned and transferred to living plants, expanding the breadth of genetic resources to include those of the past.

Training in plant genetic resources

In terms of the relevance of plant genetic resources in the context of the Environment Research and Management (ERM) group at the University of Bir-

mingham, there is a long tradition of work in this area during the past 21 years. In this period, students from more than 70 countries have received training in the theory and practice of plant genetic resources. The application of biotechnology to genetic conservation is an important aspect of this training programme, which is supported by the International Board for Plant Genetic Resources. Consequently the University of Birmingham is at the forefront of global efforts to conserve these plant resources for the future welfare of mankind.

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