Plant and algal cryopreservation: issues in genetic integrity, concepts in cryobionomics and current applications in cryobiology

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Abstract. Cryopreservation plays a vital role in the long-term in vitro conservation of essential biological material and genetic resources. Cryogenic storage techniques exist for over 100 higher plant species and numerous strains of algae. Often this multi-stage process comprises: pre-growth, cryoprotection, storage in liquid nitrogen, thawing, cell re-growth and plant regeneration. Cryopreservation involves the exposure of in vitro cells or tissues to physical, chemical and physiological stresses causing cryoinjury. Although, the effects of cryoinjury upon the genome are often unknown, any accumulative DNA polymorphisms may not be induced by cryopreservation per se but result from the whole culture-cryoprotection-regeneration process. Successful cryopreservation is often judged by cell and tissue survival and the ability to re-grow or regenerate into complete plants or form new colonies. It is desirable to assess the genetic integrity of recovered germplasm to determine whether it is ‘true-to-type’ following cryopreservation; this can be undertaken at the phenotypic, histological, cytological, biochemical and molecular level. The relevance of these approaches to examine genetic integrity will be discussed along with their limitations. The concept of ‘Cryobionomics’ is presented to re-define the idea of genetic stability regarding the re-introduction of cryopreserved organisms into the environment.

Keywords: Cryobiology; Cryopreservation; Cryoprotectants; Algae.

INTRODUCTION

Cryogenic storage at ultra-low temperatures in liquid nitrogen is highly effective for the long-term in vitro conservation of phyto-diversity - a term that includes both plants and algae (Benson, 2004). Cryopreservation has been developed using methodologies that include rapid and controlled rate cooling, colligative cryoprotection and vitrification (chemical cryoprotectants, droplet vitrification, encapsulation-dehydration and desiccation). These techniques have been applied to many algal taxa (Day, 2004) and >100 plant species using diverse cell and tissue types: cell cultures and suspensions, calluses, apices, somatic and zygotic embryos. The cryogenic process typically comprises multiple-stages ranging from tissue culture, pre-growth, acclimation, cryoprotection (colligative or vitrification), freezing (cooling), thawing (re-warming), recovery (i.e. algal re-growth), cell division and plant regeneration (Harding et al., 2004). Higher plant cells are totipotent and able to regenerate into whole plants, while microalgae typically display physiologically unique growth characteristics (Day, 2004). Successful cryopreservation is most often judged by the survival of algal cells and plant tissue cultures and their re-growth as viable cultures or complete plant regeneration. This paper highlights the approaches to assess genetic integrity and in vitro instability within the concept of ‘Cryobionomics’ and European frameworks for the cryopreservation of phyto-diversity (Harding, 2004; Harding et al., 2005).

European Algal-Plant Cryopreservation Perspectives. The European Commission has provided the resources to support plant and algal conservation projects that utilize in vitro conservation, cryopreservation technologies that include assessments of genetic stability (Harding et al., 2005). COBRA is the acronym for the project Conservation of a Vital European Scientific & Biotechnological Resource: Micro-Algae and Cyanobacteria (www.cobra.ac.uk) funded by the EU’s Fifth Framework Programme for Quality of Life and Management of Living Resources: Research Infrastructures Biological Collections (project: QLRT-2000-01645). The overall aim of COBRA was to develop a unique European biological resource centre based on existing algal culture collections. CRYMCEPT is the acronym for establishing CRYopreservation Methods for Conserving European PlantT Germplasm Collections (www.agr.kuleuven.ac.be/dtp/tro/crymcept/) funded by the EU’s Fifth Framework Programme for Quality of Life and Management of Liv-

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Where 'working practices' are

- important are those methods that detect 'non-Mendelian'
- analyses may utilise nucleic acid hybridisation or Polymerase Chain Reaction (PCR) techniques
- are unknown and may be attributed, in part to tissue culture, resulting in somaclonal variation (Harding, 1996). Although this phenomenon is less well understood in lower plants and microalgae this does not preclude its morphological and genetic manifestations. As a consequence of cryopreservation, re-growth in surviving algal-plant cultures may be subject to stresses that produce distinct differences in their genotype/phenotype profiles. There are also concerns related to cell-selection arising from the long-term culture of microalgae undergoing repeated serial subculture (Müller et al., 2007). Likewise, an important application of in vitro conservation is the use of differentiated explants comprising organised structures (shoot-tips, roots, embryos) genetically programmed to develop into 'true-to-type' plants. If precautions are taken to avoid dedifferentiation, it is generally recognised that genetic variation in regenerating plants is minimal. Obviously, the potential for genetic instability has significance for all in vitro conservation technologies. It is particularly, important to assess if germplasm surviving cryogenic storage is genetically identical to its donor material prior to cryostorage. Therefore, there is an increasing need to determine if cryopreserved germplasm is 'true to type' and to measure the extent of the near 'normal phenotype' and degree of closeness to the 'true' parental genotype.

Genetic Stability Assessments. Cell and tissue culture manipulations have an essential role in cryopreservation and their routine application presents issues regarding the genetic integrity of cultures during long-term maintenance for research and other related activities (Harding et al., 1997). There are many challenges regarding the detection of genomic change at the phenotypic, cytological and biochemical levels (Harding, 2004; Harding et al., 2005, Johnston et al. 2006; 2007a, b). While, analyses may utilise nucleic acid hybridization or Polymerase Chain Reaction (PCR) techniques to detect changes in the primary DNA sequence, equally important are those methods that detect 'non-Mendelian' epi-genetic variation in chromatin and changes in the DNA methylation of gene sequences that have altered patterns of expression (Harding, 1996; Harding et al. 2000; Johnston et al., 2005). Other considerations in the use of these analytical techniques are the amenability of biological material to examination, frequency of mutation, number of samples, genomic structure and limits of detection (Harding, 1999).

Genetic Integrity Issues. Since the early reports (~1977) of genetic stability, the literature is overall 'positive' regarding stability after cryopreservation (Harding, 2004). However, there is no single analytical technique universally applicable to all biological material and choice largely reflects end-user preferences (availability, expertise, cost, ease-of-use, reliability etc.). Although, there are advantages and limitations of the various approaches used to assess genetic stability (Harding et al., 2005). Over the last decade, less focus has been placed on phenotypic, morphological, histological, cytological and biochemical applications, and more emphasis on the use of molecular 'tools' to assess genetic stability. Regarding the 'state-of-the-art' applications, advancements in molecular biology may be described as being 'technique-driven' but they do not necessarily guarantee adequate answers to the question of genetic stability. Implicit to this analysis, is the assumption that the chosen molecular biological technique produces a DNA fragment profile that is linked to instability that is marker-assisted-selection for instability. A given molecular technique often examines only a fraction of the total genome, typically for plants with large (~109base pairs) genome sizes this is ~0.001% of the genome (Harding, 2004). It is possible, following molecular analysis of plants and algae recovered from cryopreservation, that DNA fragment polymorphic profiles may not detect genetic instability but more likely 'stability' in selected sequences. There is a need for guidance before the selection and application of specific techniques. Guidelines do exist for the selection of markers and their application to assess genetic diversity but there is little assurance that these will also provide adequate information for genetic stability assessments.

Cryobionomics – the Concept. Where 'working practices' are satisfactory for the routine storage of biological material, challenges remain regarding the assessment of stability and the adequacy of techniques prior to the export of germplasm from ex situ genebanks or the provision of 'stable' material by culture collections. Clearly, a single analytical approach is not adequate to judge genetic stability and stability assessments may be undertaken on a case-by-case basis. Moreover, there are 'no established criteria' acceptable, that are scientifically agreed to judge genetic stability after cryopreservation that are 'approved' for use in germplasm agencies, repositories or culture collections. Although, the notion of genetic stability appears to lack an accurate description; it is generally understood that cryopreservation is a process to re-introduce organisms into the environment. Therefore, an appropriate definition to incorporate the various approaches to study genetic stability would be...
‘Cryobionomics’ - a bioscience dealing with the behaviour of cryopreserved organisms in their habitat following reintroduction into natural and agri-environments. This term may be perceived as an inter-disciplinary study requiring phenotypic, histological, cytological, biochemical and molecular biological knowledge of the organism to examine aspects of cryo-injury (cellular/biochemical damage), metabolic impairment, loss of reproductive function and temporal shifts in gene expression likely to cause disruption of normal regulatory mechanisms, growth and developmental sequences (Harding, 2004).

CONCLUSION

Cryopreservation has been applied to numerous plant species and strains of algae and contemporary advancements in cryo-conservation have been supported by several major EU-funded programmes. These programmes highlight the significance of providing genetically stable organisms following the cryopreservation of phyto-diversity. An inter-disciplinary framework ‘Cryobionomics’ has been proposed to study genetic stability within algal culture collections and plant genebanks to promote the international agreement for the release of cryopreserved materials into the environment.

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REFERENCES


