

## Preface

The alarming loss of plant biodiversity both in nature and within agricultural systems has led the plant biology community to look for alternatives to *in situ* conservation. Although cryopreservation by itself is not a panacea for the global loss of biodiversity, it is a useful tool for long-term maintenance of select plant germplasm. The development of plant cryopreservation techniques for cell cultures in 1968 has led us now, 40 years later, to the stage where cryopreservation of organized tissues is a reality.

I came to the field of cryobiology through the need to conserve crop germplasm for future generations. At the time I began the field was still young and the techniques were being applied mostly to unorganized tissues and cells. The first applications of cryopreservation to organized tissues by Dr. Kutty Kartha and Dr. Akira Sakai showed the promise of the technique for the storage of plant diversity. With this encouragement I started toward my goal of storing the unique and invaluable plant germplasm at the USDA Agricultural Research Service's National Clonal Germplasm Repository, Corvallis, Oregon. I started my studies with the initial guidance of Dr. Bernard Finkle and picked up tips along the way from Dr. Lyndsey Withers, Dr. Akira Sakai and Dr. Jean Dereuddre.

While long-term storage of clonally propagated plants or those with recalcitrant seeds was once a dream, that dream became a reality by the mid 1990s. Cryopreserved collections, now located in several countries around the world, are a testament to the utility of cryopreservation. Now the challenge is to expand the utility of these techniques by making them available to laboratories that do not specialize in cryopreservation, but rather wish to use it as a safe backup for valuable plant materials.

The availability of well-tested and widely-used protocols makes the development of a book of this type possible. The first cryogenic technique, "controlled rate cooling" (also called slow cooling and two-step cooling), was the only available protocol for many years. This technique is very successful for a wide range of plant materials and is widely used for callus and suspension cell cultures. It is also easily applicable to the shoot tips of temperate plants. With the aid of a programmable freezer, relatively large amounts of plant material can be stored at one time with little technical input. At the end of the 1980s the

development of vitrification techniques provided a second approach that is applicable even to tropical plants. Several techniques were developed, but the development of Plant Vitrification Solution number 2 (PVS2) by Dr. Akira Sakai, led to the wide use of vitrification for plant tissues. Soon thereafter encapsulation dehydration was developed in the laboratory of Dr. Jean Dereuddre. This technique is also widely used and highly successful for a wide range of plants. Modifications of all these techniques are available as well. With the wide choice of techniques available it should be possible to store most types of plants.

The choice of a technique for storing a particular plant should be based on several factors. Laboratories that wish to store an occasional plant or a tropical plant will choose vitrification or encapsulation-dehydration techniques. These protocols require little more than a standard tissue culture laboratory. The techniques can be adapted to the plant material with a few simple experiments. Facilities with large amounts of temperate plant materials may wish to use controlled rate cooling to more efficiently store larger quantities of plants at one time.

This book was developed to aid in the use of cryopreservation techniques throughout the world, for the conservation of all forms of plant biodiversity. It is hoped that this volume will provide the step-by-step instructions needed to transfer cryopreservation technology to general plant biology laboratories that might make use of these protocols to store important plant materials. Often, published techniques are difficult to interpret and apply in a laboratory that is not familiar with cryopreservation. The protocols presented in this volume were tested on a range of genotypes and should be suitable for storing additional materials. By using the complete and tested protocols presented here, laboratories will have a starting point and may only need to make slight modifications before storing their valuable plant materials.

This volume was written for those active in cryobiology, and also for those who are not cryobiologists, but in need of a long-term storage method. The volume is divided into two parts. The first section introduces the reader to cryopreservation and the main techniques used. The second combines literature reviews of plant groups with defined step-by-step protocols. It is hoped that these techniques will be directly useable by the scientific community.

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