MedCrave
Step into the World of Research

Horticulture International Journal

Mini Review

Open Access

CrossMark

High throughput *in vitro* production of somatic embryos with innovative cryo cell banking system

Abstract

ArborGen Inc. was originally created as a partnership for forestry research in 2000 by West Rock (MWV), International Paper and Rubicon (Fletcher Challenge). It now produces the largest number of both conventional and advanced genetic tree seedlings in the world. Among its advanced genetics offerings, varietals have the highest level of genetic traits in yield and timber quality such as height, diameter growth, disease resistance and etc. To scale up varietals, in vitro process named somatic embryogenesis (SE) is used as a mean to mass propagate SE seedlings.

Over the years of R&D efforts, we recognized that a key challenge for scale-up is the lack of a stable and predictable bioprocess platform. The primary causes that impact stability and predictability include a challenge to receive consistent supplies of good starting cell materials, the risk of somaclonal variation due to long term exposure tomaintenance media containing hormones and risk of contamination or loss from environmental fluctuations. Accordingly our SE R&D program has been targeted to develop a robust bioprocess platform to reduce these variabilities. One of our major achievement is the development of a cell banking (CB) system, which provides high quality starting cell materials for SE production. The cell banking system includes the creations of research cell banks (RCB) and master cell banks (MCB). After the cell banks are created, the banked cells are tested and validated for their qualification for embryo production.

Currently, we successfully integrated the cell banking technology into somatic embryo production in a number of economically important tree species and crops, such as pine, spruce, sweetgum, sugarcane, avocado and banana. With the SE&CB platform, the somatic embryo & somatic seedling yields are consistent, predictable and are produced cost-efficiently on a commercial scale.

Keywords: somatic embryogenesis, cryopreservation and cell bank

Volume 6 Issue 3 - 2022

Weiming Wang, La Shaun Sanders, Alice Perry, Ebonee Duncan
ArborGen Inc., Ridgeville, South Carolina, USA

Correspondence: Weiming Wang, ArborGen Inc., Ridgeville, South Carolina, USA, Email wxwang@arborgen.com

Received: August 31, 2022 | **Published:** September 14, 2022

Introduction

New variety development and producing somatic embryos seedlings require high efficiency processing that delivers consistent and low cost products. At Arboren, this process includes five (5) technology components (Figure 1): 1) SE culture initiation where new SE cell lines are generated; 2) cryopreservation of newly generated cell lines, or research cell banks, a process that allows maintenance of cell juvenility; 3) cell line testing in lab or field to select winners; 4) cryopreservation of selected cell lines, or master cell bank creation and validation, a process allows consistent supplies of cells for SE seedling production; 5) high throughput platform that is now available for scale up production of commercial pine varieties.Among them, we believe that cryopreservation and cryo cell banking are core technology competencies that lead to success in in vitro operations such as developing and marketing new varieties.

Cryo cell banking

Cell banking[1,2] is a process by which a specific type of cell is replicated, tested and stored for research and production purposes. Cell Banking involves: 1) cell bank creation, and 2) testing/validation. Key advantages include (but are not limited to):

a. It eliminates cultural aging and deterioration. As cells get older, they tend to become less embryogenic. Without cryopreservation, it would be difficult to maintain embryogenicity.

b. It may reduce the risks of phenotypic and genotypic drift and damage due to continuous in vitro subcultures.

c. It provides indefinite, low cost storage

d. It allows flexibility for new variety testing, since cells can be stored during testing

e. Each cell bank can be extensively quality controlled and tested before they are used, enhancing reliability and reproducibility.

Cryopreservation is a technology for creating cell banks. In ArborGen, we use the controlled rate cooling method and vitrification method. The cell banking process begins with the creation of a Research Cell Bank (RCB) into the creation of a Master Cell Bank (MCB).

Creations of RCBs begin with the induction of embryogenic tissues from somatic explants. It is possible to generate many clones with different genotypes. Embryogenic tissues of each clone are cryo-preserved in about 5 -10 cryovials to create multiple RCBs. Meanwhile, cells of each RCB are cultured in the lab to produce SE seedlings, then planted in the field for testing. Years later, the clones with the best performance are selected and come to the market as a new cultivar or variety.

To market a new variety, it is essential to create a master cell bank. A MCB is an expansion of cells from a selected clone or RCB dispensed into a large quantity of cryo vials (100 – 500 vials) and stored in low temperature condition, usually -80°C or in liquid nitrogen. MCB is used to directly provide cells for SE seedling production. The MCB forms the foundation of in vitro production, as all future SE seedling production performance is based on the quality of these banks. That is why we do cell bank testing and validation; the objectives are to

confirm cell bank purity, cell viability and productivity. Purity test is performed to make sure the cell bank is contamination free. Cell viability tests ensure that cells are mostly survived cryopreservation, ideally 50% or higher. The growth recovery test verifies that the cell growth is recovered within 2-6 weeks. And a set of productivity restoration tests are performed to ensure the cell function restorations in SE tissue bulk up, embryogenicity and embryo germination.

Cell banking technology has first implemented insomatic embryo production process in pine species (Figure 2). In the last several years, the technology has also been successfully applied to other economical important crops such as avocado (Figure 3), sugarcane, banana and etc. Nowadays at ArborGen, all pine varietal seedlings come from validated MCBs. Millions of SE seedlings are produced each year and sold in US, Brazil, New Zealand and Australia. Meanwhile, continuous efforts are made each year to develop new cell lines, RCBs, field testing and then new varieties.

Process automation and mechanization

Creations of master cell banks secured the supplies of starting cell materials. Then automation and mechanization are key components for scale up. Throughout the pine SE production platform at ArborGen, 4 out of 5 main processes are automated (Figure 4). All machines for the automation are proprietary knowledge of ArborGen and were designed by ArborGen scientists.[3]

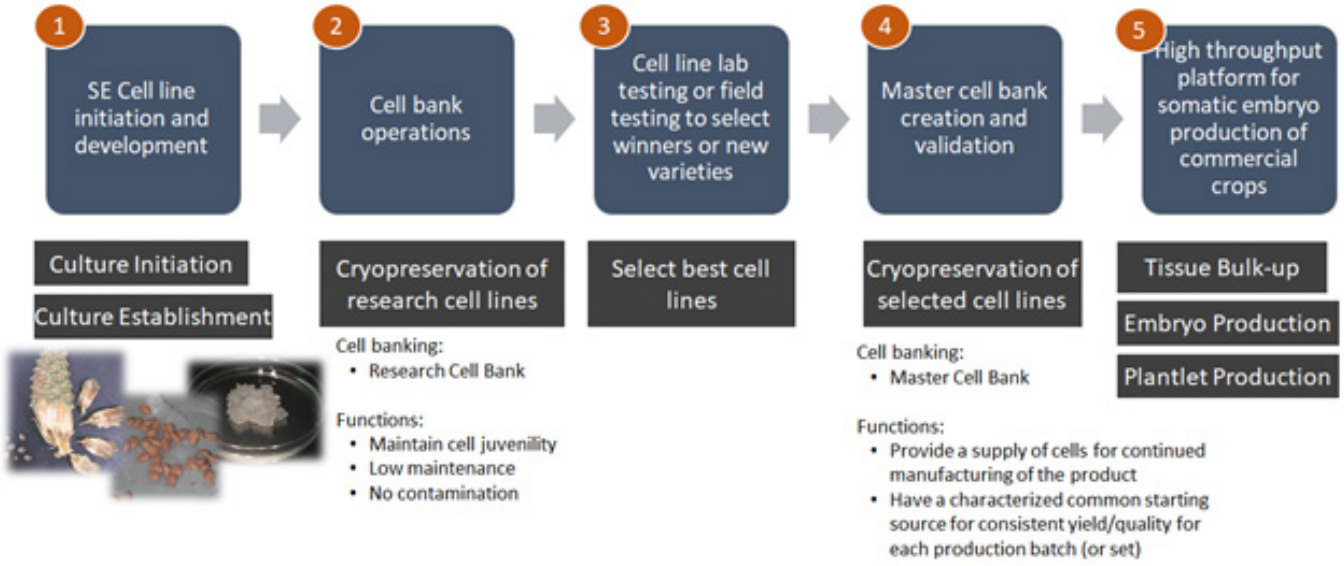

Figure 1 Flowchart of in vitro technologies with Innovative Cell Banking technology.

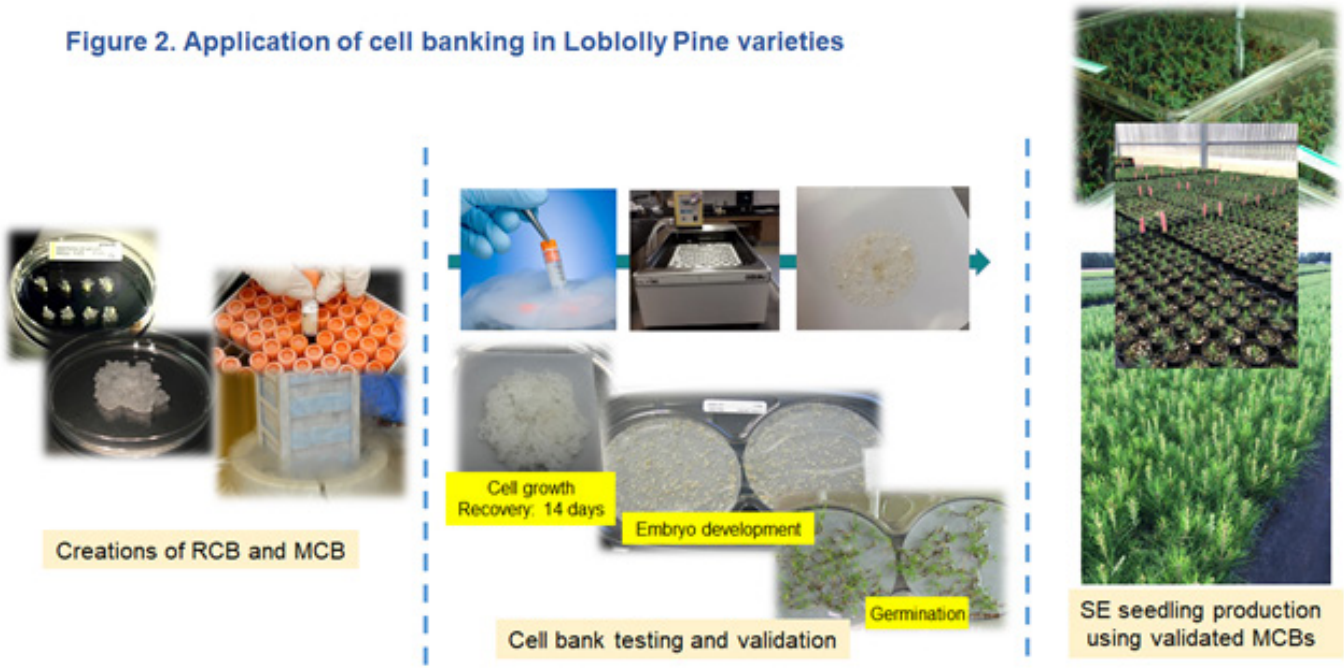

Figure 2 Application of cell banking in Loblolly Pine varieties.

Citation: Wang W, Sanders LS, Perry A, et al. High throughput *in vitro* production of somatic embryos with innovative cryo cell banking system. *Horticult Int J.* 2022;6(2):157–160. DOI: 10.15406/hij.2022.06.00259

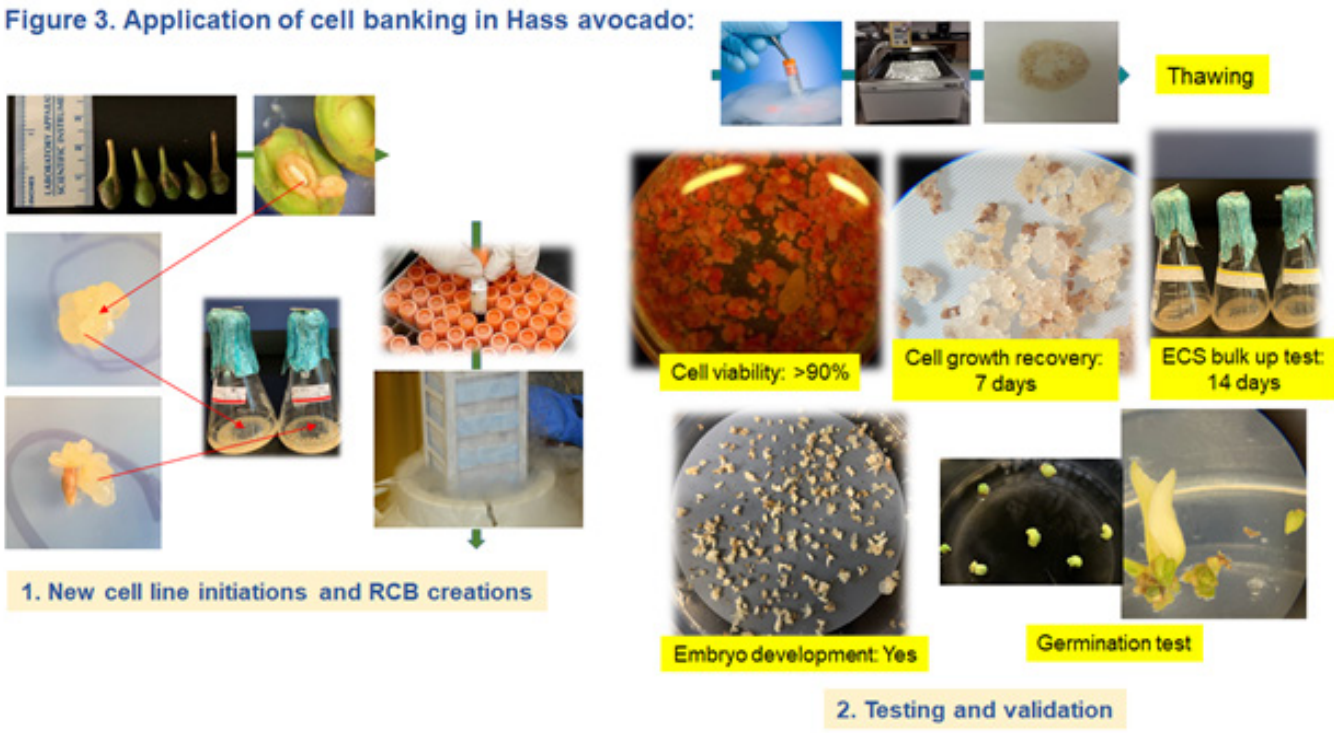

Figure 3 Application of cell banking in Hass avocado.

Figure 4 ArborGen's SE production platform with automated processes.

The first automated process uses a machine called Automated Tissue Plater, which automatically handles media vessels and dispenses embryogenic tissues to the media via a computer controlled pump and robot arm. Then, Mega Mass Harvester (MMH) is used to harvest embryos. This machine automatically performs a wash and rinse cycle to prepare embryos for the next step.The third machine is called EDWARD which stands for Embryo Dispersion With Automatic Rinse and Dry. It is used to prepare embryos for aconditioning treatment. The last automated process was designed for embryo germination. The machine is called Singulator, which automatically feed media vessels, pick up embryos and sow to the germination vessel in an evenly spaced row. Advantages to the automation of these processes are reduced labor, a significant reduction in contamination, improved embryo quality, improved germination yield and high throughput lab operation.

Citation: Wang W, Sanders LS, Perry A, et al. High throughput *in vitro* production of somatic embryos with innovative cryo cell banking system. *Horticult Int J.* 2022;6(2):157–160. DOI: 10.15406/hij.2022.06.00259

Conclusion

In conclusion, our work demonstrates the feasibility of using cell banking technology in scale up micropropagation. It provides a template that can be replicated in other crops. The use of the system has numerous advantages, and we encourage researchers to invest in this opportunity to improve in vitro propagation outcomes and facilitate new variety of commercialization.

Acknowledgments

None.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

1. Carla Benelli. Plant Cryopreservation: A Look at the Present and the Future. *Plants*. 2021;10:2744.
2. Sergio Ochatt, Maurizio Lambardi, Bart Panis, et al. Cryopreservation and In Vitro banking: a cool subject – Preface from the editors. *Plant Cell Tiss Organ Cult*. 2021;144:1–5.
3. Narender Nehra, Mark Rutter, John Clark, et al. Somatic embryogenesis and embryo harvesting and method and apparatus for preparing plant embryos for plant production. *United States Patent 7665243*. 2010.