



REVOLUTIONARY ADVANCES IN PLANT CRYOPRESERVATION: SECURING BIODIVERSITY FOR FUTURE GENERATIONS

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Introduction

In a world facing increasing environmental challenges and loss of biodiversity, the conservation of plant species is more crucial than ever before. Traditional methods of conservation have their limitations, but recent advancements in plant cryopreservation techniques offer a promising solution to safeguard the genetic diversity of plant species for future generations. This article explores the innovative strategies and technologies that are revolutionizing plant conservation efforts worldwide.

Plants play a fundamental role in sustaining life on Earth. They provide us with food, medicine, and oxygen, and contribute to the overall health of ecosystems (Timmis et al., 2021). However, due to factors such as deforestation, climate change, and habitat destruction, many plant species are facing the threat of extinction. According to the International Union for Conservation of Nature (IUCN), nearly 20% of plant species are at risk of disappearing.

The rapid loss of biodiversity due to climate change, habitat destruction, and other anthropogenic factors underscores the urgent

need for effective conservation strategies (Reid et al., 2019). Conservation efforts aim to protect and preserve plant species to maintain ecosystem balance and ensure the availability of resources for future generations. Traditional methods, such as seed banks have been valuable in preserving plant diversity and it has limitations, especially for species with recalcitrant seeds that do not survive conventional drying and storage.

These methods are not always sufficient for species that are difficult to store using traditional means, such as recalcitrant seeds or vegetatively propagated plants. Cryopreservation offers a viable alternative by preserving genetic material in a state of suspended animation, allowing for the long-term conservation of plant tissues, seeds, and even entire embryos. In the realm of biological preservation, the innovative techniques of plant cryopreservation are making headlines.

Cryopreservation, the process of freezing biological materials at extremely low temperatures to maintain their viability, is not a new concept. However, recent advancements have propelled this technique to new heights, offering groundbreaking

solutions for the conservation of plant biodiversity. These innovations promise to safeguard a wide array of plant species, from crops to endangered wild flora, ensuring their survival for generations to come. Cryopreservation is a technique that involves storing biological material at ultra-low temperatures, typically in liquid nitrogen, to halt biological processes and preserve the material for an extended period. While cryopreservation has been used for decades in preserving animal cells and tissues, its application to plant conservation has been more recent and challenging due to the complex nature of plant cells. Recent advancements in plant cryopreservation techniques have overcome many of the challenges associated with preserving plant genetic material.

One of the key breakthroughs is the development of vitrification, a method that involves rapid freezing of plant tissues to prevent the formation of ice crystals that can damage cells. Vitrification has proven to be highly effective in preserving a wide range of plant species, including orchids, bananas, and endangered tree species (Coelho et al., 2020).

Understanding Vitrification in Plant Cryopreservation:

Vitrification is a cutting-edge cryopreservation technique that involves ultra-rapid cooling of plant tissues to cryogenic temperatures, typically below -196°C , in the presence of cryoprotectants to prevent the formation of ice crystals. Unlike slow freezing methods, which can lead to ice crystal formation and cell damage, vitrification ensures the preservation of cellular structures by transforming the intracellular solution into

a glass-like state without crystallization (Fonseca et al., 2016). The success of vitrification in plant cryopreservation lies in its ability to maintain the viability and genetic integrity of plant cells during the freezing and thawing process. By avoiding ice crystal formation, vitrification minimizes cellular damage and enables the long-term storage of plant tissues, seeds, and embryos without compromising their viability or genetic stability.

Applications of Vitrification in Plant Conservation:

Vitrification techniques have revolutionized plant conservation efforts by providing a reliable and efficient method for preserving a wide range of plant species, including recalcitrant seeds, delicate orchids, and endangered tree species. The versatility of vitrification allows conservationists and researchers to store plant genetic material for extended periods, ensuring the survival of rare and valuable plant species for future generations. One of the key applications of vitrification in plant conservation is the establishment of cryopreserved gene banks, where plant tissues and seeds are stored at ultra-low temperatures for long-term preservation.

These gene banks serve as invaluable repositories of plant genetic resources, offering a safeguard against biodiversity loss and providing a source of material for research, breeding programs, and restoration efforts. Vitrification techniques have also been instrumental in conserving plant species with limited seed availability or those that are difficult to store using traditional seed banking methods. By preserving plant tissues, shoot

tips, and embryos through vitrification, conservationists can maintain the genetic diversity of rare and endangered plants, protect against genetic erosion, and support efforts to reintroduce species back into their natural habitats.

Exploring V-Cryo Plate Technique:

The V-Cryo Plate technique is a novel cryopreservation method that involves the use of a specialized cryo-plate with a unique V-shaped design. This innovative approach enables plant tissues or embryos to be placed in the V-shaped wells of the cryo-plate, allowing for efficient cooling and storage at cryogenic temperatures. The V-Cryo Plate technique offers several advantages over traditional cryopreservation methods, including increased surface area for sample loading, improved cooling rates, and enhanced uniformity of cooling across samples. One of the key benefits of the V-Cryo Plate technique is its scalability and versatility, making it ideal for high-throughput cryopreservation of plant materials. Researchers and conservationists can use V-Cryo Plates to store a large number of plant samples simultaneously, streamlining the cryopreservation process and facilitating the conservation of diverse plant species with varying storage requirements.

Moreover, the V-Cryo Plate technique has been successful in preserving a wide range of plant species, including recalcitrant seeds, delicate tissues, and vegetatively propagated plants. By leveraging the unique design and functionality of V-Cryo Plates, conservationists can ensure the long-term viability and genetic integrity of plant materials, contributing to the conservation of plant biodiversity and the restoration of degraded ecosystems.

Unveiling D-Cryo Plate Technique:

In addition to the V-Cryo Plate technique, the field of plant cryopreservation has witnessed the emergence of the D-Cryo Plate technique, another innovative approach to preserving plant genetic material at ultra-low temperatures. The D-Cryo Plate technique features a dual-sided cryo-plate design with two distinct cooling surfaces, allowing for more efficient cooling and storage of plant samples. The D-Cryo Plate technique offers enhanced cryopreservation capabilities by providing different cooling rates on each side of the cryo-plate, catering to the specific requirements of diverse plant species and tissues. This dual-sided design enables researchers to optimize cryopreservation protocols, improve sample viability, and enhance the overall success rate of plant preservation efforts. Furthermore, the D-Cryo Plate technique has proven to be effective in preserving challenging plant materials, such as recalcitrant seeds, shoot tips, and somatic embryos. By leveraging the advanced cooling capabilities of D-Cryo Plates, conservationists can overcome the limitations of traditional cryopreservation methods and expand the scope of plant conservation efforts to include a broader range of species and genetic resources.

Understanding Differential Scanning Calorimetry in Plant Cryopreservation:

Differential Scanning Calorimetry is a sophisticated analytical technique used to study the thermal properties of materials as a function of temperature. In the context of plant cryopreservation, DSC techniques are employed to analyze the thermal behaviour of plant tissues, cells, and cryoprotectant solutions during the freezing and thawing

process. By measuring the heat flow associated with phase transitions, crystallization, and melting events, DSC provides valuable insights into the effects of cryopreservation on plant samples at a molecular level. One of the key advantages of Differential Scanning Calorimetry in plant cryopreservation is its ability to assess the efficacy of cryoprotectant solutions in protecting plant cells from damage during freezing and thawing. By monitoring the thermal properties of plant tissues exposed to varying concentrations of cryoprotectants, researchers can optimize cryopreservation protocols, identify suitable cryoprotectants for specific plant species, and enhance the survival rates of preserved plant material (Benelli, 2021)

Applications of Differential Scanning Calorimetry in Plant Conservation:

DSC techniques have revolutionized plant cryopreservation efforts by providing a precise and sensitive tool for characterizing the thermal properties of plant samples subjected to cryogenic temperatures. By analyzing the thermal responses of plant tissues and cryoprotectant solutions, DSC enables researchers to tailor cryopreservation protocols to the specific requirements of different plant species, tissues, and developmental stages. One of the key applications of Differential Scanning Calorimetry in plant conservation is the evaluation of cryoprotectant toxicity and compatibility with plant tissues. Through DSC analysis, researchers can determine the optimal concentration and type of cryoprotectants that maintain cell viability and structural integrity during the freezing and thawing process. This knowledge is critical for

developing effective cryopreservation strategies that minimize cellular damage and maximize the post-thaw recovery of plant samples (Bettoni et al., 2021). Furthermore, DSC techniques have been instrumental in studying the effects of cryopreservation on the thermal properties of plant tissues, providing valuable insights into the mechanisms underlying cell injury, ice formation, and cryoprotectant interactions. By elucidating the thermal behaviour of plant samples during cryopreservation, researchers can refine preservation protocols, troubleshoot issues, and enhance the overall success of plant conservation efforts.

Applications and Implications

The implications of these advanced cryopreservation techniques are far-reaching. In agriculture, they offer a lifeline for the conservation of crop genetic diversity, which is essential for breeding programs and food security. By preserving the genetic material of wild relatives of crops, scientists can tap into a reservoir of traits such as disease resistance and drought tolerance, which are vital for developing resilient crop varieties in the face of climate change (Naqvi, et al., 2020).

In the realm of environmental conservation, cryopreservation provides a means to protect endangered plant species that are on the brink of extinction. By storing seeds, tissues, or embryos, conservationists can ensure the survival of these species even if their natural habitats are lost. Moreover, cryopreservation facilitates the restoration of damaged ecosystems by providing a source of plant material for reintroduction and habitat rehabilitation efforts.

Future Prospects and Challenges

Despite the remarkable progress, plant cryopreservation still faces several challenges. One of the primary obstacles is the species-specific nature of cryopreservation protocols. Each plant species may require tailored procedures to achieve optimal preservation and recovery. Consequently, ongoing research is focused on understanding the underlying mechanisms of cryo-injury and developing universal protocols that can be broadly applied across different species. Another challenge lies in the long-term storage and monitoring of cryopreserved material. Ensuring the stability and integrity of preserved specimens over extended periods requires meticulous management and advanced cryogenic facilities. Collaborative efforts and investment in infrastructure are essential to build and maintain global cryopreservation repositories.

Conclusion

The advancements in plant cryopreservation techniques herald a new era in the conservation of biodiversity. By leveraging cutting-edge methods such as vitrification, encapsulation-dehydration, and cryo-plate techniques, scientists are making significant strides in preserving the genetic heritage of plants. These innovations not only secure the future of agriculture and food security but also play a crucial role in protecting our planet's invaluable natural heritage. As research continues to evolve, the promise of cryopreservation as a cornerstone of biodiversity conservation becomes increasingly attainable, offering hope for a sustainable and resilient future.

References:

1. Benelli, C. (2021). Plant cryopreservation: A look at the present and the future. *Plants*, 10(12), 2744.
2. Bettoni, J. C., Bonnart, R., & Volk, G. M. (2021). Challenges in implementing plant shoot tip cryopreservation technologies. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 144(1), 21-34.
3. Coelho, N., Gonçalves, S., & Romano, A. (2020). Endemic plant species conservation: Biotechnological approaches. *Plants*, 9(3), 345.
4. Fonseca, F., Meneghel, J., Cenard, S., Passot, S., & Morris, G. J. (2016). Determination of intracellular vitrification temperatures for unicellular microorganisms under conditions relevant for cryopreservation. *PLoS One*, 11(4), e0152939.
5. Naqvi, R. Z., Siddiqui, H. A., Mahmood, M. A., Najeebullah, S., Ehsan, A., Azhar, M., ... & Asif, M. (2022). Smart breeding approaches in post-genomics era for developing climate-resilient food crops. *Frontiers in Plant Science*, 13, 972164.
6. Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological reviews*, 94(3), 849-873.
7. Timmis, K., & Ramos, J. L. (2021). The soil crisis: the need to treat as a global health problem and the pivotal role of microbes in prophylaxis and therapy. *Microbial Biotechnology*, 14(3), 769-797.