Frosting the Future: Cryobiotechnology's Impact in Agricultural Innovation

Conchita, S.[1], Priyadarshini, S.[2]and Krishna Kumar, S[3].
[1] Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bengaluru-560065.
[2] Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru-560065.
[3] Department of Agricultural Entomology, University of Agricultural Sciences, GKVK, Bengaluru-560065.
Corresponding Author : conchitasomu@gmail.com

Manuscript No: KN-V2-01/008

Introduction:

Habitat loss due to human activities like deforestation and urbanization is shrinking the natural spaces where plants thrive, leading to a significant loss of genetic diversity. Climate change exacerbates this issue by altering temperature, precipitation patterns and habitats, forcing plants to adapt or migrate. The intersection of plant habitat loss and climate change poses a critical threat to genetic diversity, jeopardizing the survival of numerous plant species. This loss of genetic variation diminishes the resilience of plant populations, making them more susceptible to diseases, pests, and environmental stresses, ultimately threatening their survival. However, conservation technologies offer promising solutions to mitigate this crisis by preserving habitats and implementing strategies to safeguard plant diversity. Advanced preservation techniques like cryopreservation and seed banking enable the long term storage of diverse plant genetic material, safeguarding against extinction. Additionally, innovative breeding programs and genetic engineering techniques can potentially enhance the resilience of plants against changing climates, contributing to the preservation of their genetic diversity in the face of environmental challenges.

What is cryobiotechnology and its usage?

Cryobiotechnology is the modern field of study which involves the response by biological organisms when exposed to extreme low temperatures in natural or artificial conditions. This field of study is very significant in modern techniques like cryopreservation of organisms, tissues, cells which are used in agriculture, industry, medicine and conservation practices (Pritchard, 2018).

Cryobiotechnology mainly utilizes cryopreservation i.e., storage of cells or tissues in liquid nitrogen (LN) (-196°C) or in its vapor phase (-156°C). It is a very important process in preserving the genetic diversity of many organisms for long term storage. In these ultra low temperatures, the cell stops it cell division, metabolic and other biochemical reactions, thus preventing the genetic changes during long term storage. Storing genetic material in gene banks through in vitro cultures, while advantageous, can be costly and threatened with issues like somaclonal variation and contamination loss. Cryopreservation is considered a more promising alternative method. Several researches have been conducted to prove that cryopreservation is an essential tool to store and conserve plant germplasm which is needed for agriculture and also to store desiccation tolerant seeds, pollens, zygotic embryos, dormant buds, spores, shoot tips, somatic embryos algae and gametophytes for a long term i.e., for nearly 2-3 decade. Cryobiotechnology is necessary for conserving wide range of plant species that cannot be conserved in conventional seed banks.

Apart from agricultural species, many ornamental species are also cryopreserved as a valuable breeding material source and the first report was on Dianthus hybrida. Even cryopreservation has been carried out for horticulture woody plants like apple and pear plants. Cryo-banks to preserve partially desiccation tolerant seeds of Citrus and Coffee seeds have been established in France, Brazil, and Central America (Krishnan, 2013)

Types of cryopreservation:

Cryopreservation techniques for plant cells consists conventional and novel methodologies. Traditional

approaches involve gradual freezing employing cryoprotectants such as glycerol, dimethylsulphoxide (DMSO), sucrose and ethylene glycol. The specimens undergo controlled cooling at rates of 0.3-0.5°C/min until reaching -40°C and then are rapidly immersed in liquid nitrogen (Kumu et al., 1983).Contrarily; modern methods encompass various techniques including vitrification, encapsulation vitrification, encapsulation dehydration, droplet vitrification and cryo-plates.

Vitrification is the process which involves the formation of glassy structures from solutes present intracellularly. The vitrification solution effectively eliminates freezable water within the cell, followed by rapid ultra-freezing through immersion in liquid nitrogen. Notably, PVS2, comprising 30% glycerol, 15% ethylene glycol, 15% DMSO dissolved in 0.4M sucrose, demonstrates reduced toxicity towards plant cells compared to other solutions (Jiroutova and Sedlák 2020).

Encapsulation vitrification and encapsulation dehydration methods includes enclosing plant tissues or cells in alginate beads, subsequently dehydrating them using concentrated vitrification solutions or physical means like silica gel or laminar airflow, respectively. Droplet vitrification involves pre-treating shoot tips with a loading solution containing 2M glycerol and 0.4 M sucrose to induce osmo-tolerance, followed by exposure to vitrification solution and immersion in liquid nitrogen after placement on aluminum foil strips.

The latest innovation, cryo-plate technology, involves keeping samples in small wells of cryo-plates for subsequent dehydration using vitrification solutions or physical desiccation, followed by immersion in liquid nitrogen. This method has advantages in easy sample handling and user friendliness. Each technique in cryopreservation presents distinct merits, collectively contributing to the long-term preservation of plant cells. Among, all these methods, vitrification is the most commonly employed approach for plant material because of its simplicity and applicability to large germplasm collections. (Jiroutova and Sedlák 2020)

Cryotherapy:

Cryobiotechnology involves another new technique called cryotherapy which helps in eradication of disease causing pathogens by immersing the infected plant part in liquid nitrogen. This technique was first reported by Brison et al., 1997 in plum by eradicating plum pox virus from in-vitro grown shoot tips. Till date, cryotherapy is employed in different plant species like potato, sweet potato, banana, raspberry, grapevine and apple and mainly, viruses, bacteria and phytoplasma are eradicated in these plant species.

Cryotherapy targets plant shoot tips with unique meristematic cells which are resistant to freezing damage caused by ice crystal formation. Liquid nitrogen treatment selectively eliminates the differentiated cells and preserves meristematic cells, enabling their regeneration and the growth into new virus free plants. Cryotherapy effectively removes banana streak virus (BSV), cucumber mosaic virus (CMV), grape virus A (GVA), potato leaf roll virus(PLRV) and potato virus Y (PVY) infecting differentiated cells, but viruses affecting meristematic cells, like raspberry bushy dwarf virus (RBDV), pelargonium flower break virus (PFBV) and pelargonium line pattern virus (PLPV), is more challenging. Combining cryotherapy with thermotherapy inhibits virus movement toward meristematic cells, thereby enhancing the virus elimination. (Jiroutova and Sedlák 2020)

Another breakthrough preservation technique is to preserve obligate pathogens like viruses, viroids and phytoplasmas for basic research, genetic transformation, designing of nanodrugs, to develop plant based vaccines and also for antigen preparation (immunological detection assays). All these applications make it necessary to maintain key collections of the pathogens under in-vitro condition (Zhao et al., 2019)

Conclusion:

In conclusion, cryobiotechnology, encompassing cryopreservation and cryotherapy, stands as a groundbreaking tool in preserving and manipulating biological systems. Cryopreservation offers a means to safeguard genetic diversity, conserve endangered species, and store valuable cells, tissues, and organs for future use. On the other hand, cryotherapy, leveraging extreme cold to selectively preserve resilient meristematic cells while destroying infected or differentiated cells, cryotherapy offers a pathway to generate virus-free plants in agricultural plant species. These techniques have huge potential in medicine and agriculture, offering new ways to preserve things for long-term preservation and enhanced biological resilience towards changing environmental conditions.

Reference:

Jiroutová, P. and Sedlák, J., 2020. Cryobiotechnology of plants: A hot topic not only for gene banks. Applied Sciences, 10(13), p.4677.

Kumu, Y., Harada, T. and Yakuwa, T., 1983. Development of a Whole Plant from a Shoot Tip of Asparagus officinalis L. Frozen Down to-196□. Journal of the Faculty of Agriculture, Hokkaido University, 61(3), pp.285-294.

Krishnan, S., 2013. Current status of coffee genetic resources and implications for conservation. CABI Reviews, (2013), pp.1-9.

Brison, M., de Boucaud, M.T., Pierronnet, A. and Dosba, F., 1997. Effect of cryopreservation on the sanitary state of a cv Prunus rootstock experimentally contaminated with Plum Pox Potyvirus. Plant Science, 123(1-2), pp.189-196.

Zhao, L., Wang, M., Li, J., Cui, Z., Volk, G.M. and Wang, Q., 2019. Cryobiotechnology: A double-edged sword for obligate plant pathogens. Plant disease, 103(6), pp.1058-1067.

"Pritchard, H.W., 2018. The rise of plant cryobiotechnology and demise of plant cryopreservation?. Cryobiology, 85, pp.160-161."