Journal of Advance Agricultural Research
January,2025
e-ISSN No. 3049-320X (Online)

Review Article

The Future of Plant Conservation: Biotechnological Approaches for Rare and Endangered Species

Umme Salma[1]*, Nirmal Mandal[2]

[1]Assistant Professor, Department of Agricultural Biotechnology, School of Agricultural Sciences, Sister Nivedita University, Newtown

[2]Professor, Department of Agricultural Biotechnology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur

Correspondence: umme.s@snuniv.ac.in

Abstract: The conservation and sustainable use of the diversity present in plant genetic resources and traditional knowledge of germplasm within and among plant species represent economic, scientific, and societal values that have the potential to solve the food security problems arising from our expanding global population. Advances in biotechnology fields such as in vitro culture technology, cryopreservation, and molecular markers have generated significant improvements in methods of conservation of rare and endangered plant genetic resources and the traditional knowledge of germplasm, and their valuable management, in effective ways. A strategic and forward vision for conservation of plant genetic resources and traditional knowledge of germplasm and sustainable use of plant resources in the twenty-first century is of far-reaching significance for the Earth's sustainable development.

Keywords: In vitro culture, Cryopreservation, Molecular markers, Micrografting

Received: 17/11/2024
Revised: 20/12/2024
Accepted: 21/01/2025
Published: 25/01/2025

Citation: Salma, U. and Mondal, N. 2025. The Future of Plant Conservation: Biotechnological Approches for Rare and Endangered Species. *J. Adv. Agri. Res.* :1(1): 35-47.

1. Introduction

Biodiversity is the cornerstone of ecosystem stability, and plants form the foundation of terrestrial ecosystems. However, anthropogenic activities such as habitat destruction, climate change, and overexploitation have led to the decline of many plant species, classifying them as rare, endangered, or threatened (RET). The conservation of these plants is essential not only for ecological balance but also for sustaining cultural and economic values.

More than half of the world's plant species are endemic to 34 global biodiversity hotspots (GBH), which once accounted for 15.7% of the earth's land surface but are now only 2.3%. The gradual disappearance of terrestrial natural ecosystems for various human activities has led to the extinction of many plant species worldwide. This is often because native vegetation has been cleared for agriculture, which causes erosion, salinization, and the invasion of alien species. More recently, climate change has emerged as a significant new threat. The International Union for Conservation of Nature's Red List of Threatened Plants, which was initially released in 1998 (IUCN 1998) and currently covers over 8,000 species, has several endemic species that are in danger of going extinct (IUCN 2010). The problems of preserving endangered species cannot be solved by in situ conservation alone. A considerable percentage of species are not suitable for seed banking, even if it can be used for the ex situ conservation of most endangered species. In these circumstances, it is crucial to preserve the genetic diversity of species and populations, and micropropagation can yield a significant number of propagules from a cross-section of a region's genetic diversity (Rogers 2003). International authorities must create and oversee programs for the coordinated in situ and ex situ conservation of rare and endangered plant species. Funding is frequently limited, yet botanical gardens are increasingly tasked with preserving and collecting endangered species. By establishing goals for comprehending and preserving plant diversity, encouraging sustainable usage, offering education, and developing capacity to support plant conservation, the Global Strategy for Plant Conservation (GSPC 2010), which was agreed by more than 180 nations in 2002, seeks to stop this loss. While conventional conservation methods have been effective to some extent, they face limitations in scalability, precision, and long-term viability. Biotechnology offers innovative tools and techniques to overcome these limitations, providing new pathways for conserving RET plants. This paper highlights the various biotechnological approaches used in plant conservation and their potential for addressing conservation challenges.

2. Biotechnological Tools for Plant Conservation

Tissue Culture Techniques

Micropropagation

Micropropagation enables the rapid multiplication of plants from a small amount of tissue. Techniques such as shoot-tip culture, node culture, and somatic embryogenesis are commonly used to produce large numbers of identical plants. In a short period of time and limited space, it allows the production of numerous plant species. It is exploited for the production of pathogen-free cloned plants for agriculture and forestry usages. From conservation point of view, the regenerated plantlets should have minimum somatic variations through micro-propagation method by reducing the number of

sub-culturing and axillary bud or shoot tip culture. *Echinacea purpurea*, a medicinal plant, has been propagated using micropropagation to meet conservation and commercial needs (Baskaran *et al.,* 2021). The micropropagation of *Lavandula pedunculata* was done for essential oil production (Zuzarte *et al.,* 2010) without affecting natural resources.

Somatic Embryogenesis

Somatic embryogenesis is the process by which a somatic embryo develops from one or more cells. Gene cloning and plant genetic transformation rely on plant tissue culture techniques for regeneration on appropriate conditions. However, somatic embryos, which can be derived from reproductive explants such as ovaries, stigmas, anthers, and whole flowers, have been utilized as the starting material for all transformation techniques (Kikkert *et al.,* 2005, Gambino *et al.,* 2007, Prado *et al.,* 2010). Compared to previous approaches, somatic embryogenesis has various advantages and helps produce a high number of plants regardless of the season. This technique can be used to create haploid plantlets, which may be useful in the study of mutations. In the past, producing homozygous lines through selfing presented numerous challenges for plant breeders; however, biotechnological techniques play a significant role in producing homozygous lines from gametic embryogenesis (Germana *et al.,* 2011). Somatic embryogenesis has been used to conserve the critically endangered Syzygium travancoricum tree (Thomas *et al.,* 2017).

Protoplast culture

Fusion is a physical process in which two or more protoplasts meet and adhere to one another, either naturally or with the help of substances that cause fusion. It is possible to transfer some beneficial genes from one species to another, including those that are resistant to disease, nitrogen fixation, quick growth and increased rate of product synthesis, protein quality, frost hardiness, drought resistance, herbicide resistance, and resilience to heat and cold. Thus, genes from other organisms have been combined using this biotechnological method to produce strains with the required characteristics. It is possible to create para-sexual hybrid protoplasts by experimentally fusing two genetically distinct protoplasts that were separated from the somatic cells.

Cryopreservation

With cryopreservation, tissues can be stored for a long time in cryo-tanks with liquid nitrogen (LN, -196 °C), and after being reheated, plants can grow again from these tissues (Engelmann 2011, Benson 1999, Bi et al., 2021). Explants kept in cryopreserved settings have little metabolic activity and cellular divisions, which maintains the genetic integrity for an extended period of time (Engelmann 2011, Panis and Nagel 2020; Reed 2017). Plant tissues that are frozen in liquid nitrogen must have their water content replaced with cryoprotectants, which are anti-freezing chemicals that can prevent ice

formation and preserve cellular integrity. Plant species-specific cryopreservation techniques are common (Benson 1999). Plant species with irregular seed production and those whose seed collection is restricted due to declining populations can benefit from the use of commonly used explants, such as meristems, nodes, buds, roots, and seeds (Bi *et al.*, 2021; Popova *et al.*, 2015). Plants like the golden paintbrush (Castilleja levisecta Greenm) (Salama *et al.*, 2018) and cherry birch (Betula lenta L.) (Rathwell *et al.*, 2016) have been reintroduced into their natural habitats through cryobanking. The critically endangered tissues in liquid nitrogen necessitate replacing the water content of the tissues with cryoprotectants, which are anti-freezing substances that can prevent ice formation and

Table 1: Conservation status and in vitro propagation of some threatened plant

Scientific Name	Traditional Uses	In Vitro Method Used	Reference
Castanea americana	Food, wood	Transgenic modification	Barnhill-Dilling and Delborne 2019
Turbinicarpus sp	Medicinal, ceremonial	Tissue culture	Carlín et al., 2015
Gentiana kurroo	Medicinal	Shoot culture	Sharma 2001
Eucalyptus spp	Medicinal	Tissue culture	McComb et al., 1996
Rhinacanthus nasutus	Medicinal, dye	Tissue culture	Reshi et al., 2018
Rauvolfia serpentina	Medicinal	Organogenesis	Baksha et al.2007
Withania somnifera	Medicinal	Hairyroots	Kumar et al., 2005
Artocarpus altilis	Medicinal, food	Meristem culture	Murch et al., 2007
Hordeum vulgare	Food	Embryo, cryopreservation	Fretz et al., 1992
Zea mays	Food, ceremonial	Embryo, seed cryopreservation	Usman and Abdulmalik, 2010, Perez et al., 2017
Wrightia tinctoria	Medicinal	Stem cuttings	Mridula and Nair, 2018
Betula medwdewii Regel	Medicinal, wood	Shoot tips, buds	Gaidamashvili et al., 2015a, b
Castanea sativa	Medicinal	In vitro nodal explants, Buds, Embryonic axis	Sáez et al., 2012

preserve cellular structure. Protocols for cryopreservation are frequently species-specific (Benson 1999). Plant species with irregular seed production and those whose seed collection is restricted due to declining populations can benefit from the use of commonly used explants, such as meristems, nodes, buds, roots, and seeds (Bi *et al.*, 2021; Popova *et al.*, 2015). Plants like the golden paintbrush (Castilleja levisecta Greenm.) (Salama *et al.*, 2018), cherry birch (Betula lenta L.) (Rathwell *et al.*, 2016), and the critically endangered pearl-like androcalva (Androcalva perlaria Wilk.) (Whiteley *et al.*, 2016) have

all been reintroduced into their natural habitats through cryobanking. A Canadian endangered plant, the streambank lupine (Lupinus rivularis Lindl.), has recently been successfully cryopreserved and micropropagated (Popova *et al.,* 2021). Techniques like droplet vitrification have been used for successful cryopreservation of *Dioscorea deltoidea* (Engelmann, 2011). Cryopreservation techniques have been employed to conserve *Taxus baccata*, a source of the anti-cancer compound paclitaxel (Sharma *et al.,* 2015). Mangrove species such as *Rhizophora apiculata* have been successfully propagated through micropropagation and genetic studies to restore degraded coastal ecosystems (Kathiresan & Bingham, 2001).

Micrografting

Micro-grafting, which has been researched both in vitro and in vivo, is the process of rejuvenating the mature scion shoot tip onto the juvenile root stock. In order to produce disease-free scions, rejuvenate and/or invigorate mature shoot materials, increase the possibility of cloning mature plants true to type, and investigate graft unions, this technique was created (Jonard, 1986) (Onay *et al.*, 2003). In vitro micrografting was used to regenerate the kinnow mandarian without the use of viruses (Singh *et al.,* 2008). The oxidation of phenolic chemicals causes browning of cut surfaces, which is a constraint in the creation of micrografted plantlets. Dipping the shoot scion in the MS medium, however, might solve this issue.

Genetic Engineering

Genetic engineering facilitates the introduction of specific traits to improve plant resilience or to preserve unique genetic attributes. The transformation of *Artemisia annua* for enhanced production of artemisinin, a key anti-malarial compound (Ikram *et al.,* 2017). These genetically transformed root cultures can produce secondary metabolites in large amounts comparable with those in intact plants, and the transformed root lines can be a promising source for the constant and standardized production of secondary metabolites (Kundu *et al.,* 2019).

Molecular Markers

DNA based markers either PCR based or non-PCR based provide genetic structure of the plant species and have been used in various fields such as embryology, genetic engineering, physiology, taxonomy etc. Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs), and Single Nucleotide Polymorphisms (SNPs). These markers are helpful in the identification of plant species and their cultivars to clarify errors occurred with the herbs in the local markets (Kiran et al., 2010). Molecular markers are crucial for understanding genetic diversity and identifying distinct genotypes within RET species. AFLP markers have been used to study genetic diversity in *Rauvolfia serpentina* populations (Rout et al., 2009). RAPDs have been

extensively used in assessing genetic relationship among various accessions of different plant species (Khan *et al*., 2010, Khan *et al*., 2011). SSR marker has been used for the assessment of genetic diversity in a numerous crops such as *Psathyrostachys huashanica, Zea maize, Apium graveolens*, *Prunus domestica* L., cherry plum (*P. cerasifera* Ehrh.) and sloe (*P. spinosa* L.) (Liu *et al*., 2010, Wang *et al*., 2011, Horvath *et al*., 2011).

Next-Generation Sequencing (NGS) and Genomics

NGS has revolutionized conservation genomics by enabling the sequencing of entire genomes at a reduced cost. This technology aids in identifying genes associated with stress tolerance and adaptability. Genome sequencing of *Amborella trichopoda*, an ancient angiosperm, has provided insights into evolutionary conservation (Albert *et al*., 2013). CRISPR/Cas9 allows precise modifications of plant genomes, offering potential for restoring endangered plants by improving stress resistance and adaptability. Gene editing in *Orchidaceae* species to enhance flowering and adaptability (Nishihara *et al*., 2020).

3. Conclusion and future prospects

The protection and enhancement of plant species, particularly rare and endangered ones with economic and medicinal significance, require biotechnological technologies. For plant species to survive in their natural environments, genetic diversity is essential. However, the plant's capacity to adapt to changing environmental conditions and demographic shifts may deteriorate over time if its genetic variety is lost. It is extremely difficult to restore the vast diversity of plant species that have vanished from their natural habitat since they cannot be regenerated. In order to increase the genetic diversity of rare and endangered plants, these technologies should be used in a variety of methods for the conservation and enhancement of different plant species. DNA banking, which has been implemented in a few nations, is another promising technique for the preservation of biological information by keeping genomic DNA at low temperatures. DNA isolation is simple and has several applications in the description and use of biodiversity. the application of these biotechnological instruments to endangered and rare plant species.

References

Albert, V. A., Barbazuk, W. B., Der, J. P., Leebens-Mack, J., Ma, H., Palmer, J. D., ... DePamphilis, C. W. (2013). The Amborella genome and the evolution of flowering plants. *Science*, *342*(6165), 1241089. https://doi.org/10.1126/science.1241089

Barnhill-Dilling, S. K., & Delborne, J. A. (2019). The genetically engineered American chestnut tree as an opportunity for reciprocal restoration in Haudenosaunee communities. *Biological Conservation*, *232*, 1–7. https://doi.org/10.1016/j.biocon.2019.01.018

Baskaran, P., Kumari, A., Kumar, A., & Van Staden, J. (2021). Micropropagation and secondary metabolite production in *Echinacea purpurea*. *Plant Cell Reports, 40*(2), 291-304. https://doi.org/10.1007/s00299-020-02614-4

Carlín, A. P., Tafoya, F., Solís, A. G. A., & Pérez-Molphe-Balch, E. (2015). Effects of different culture media and conditions on biomass production of hairy root cultures in six Mexican cactus species. *In Vitro Cellular & Developmental Biology-Plant, 51,* 332–339. https://doi.org/10.1007/s11627-015-9682-8

Engelmann, F. (2011). Use of biotechnologies for the conservation of plant biodiversity. *In Vitro Cellular & Developmental Biology-Plant, 47*(1), 5-16. https://doi.org/10.1007/s11627-010-9327-2

Fretz, A., Jahne, A., & Lorz, H. (1992). Cryopreservation of embryogenic suspension cultures of barley (*Hordeum vulgare* L.). *Botanica Acta, 105,* 140–145. https://doi.org/10.1111/j.1438-8677.1992.tb00291.x

Gaidamashvili, M., & Khurtsidze, E. (2015a). Development of the conditions for *In Vitro* propagation of threatened birch species (*Betula* spp.). *Bulletin of Forestry, 9,* 28–31.

Gaidamashvili, M., Khurtsidze, E., & Barblishvili, T. (2015b). Micropropagation of threatened *Betula* species for *In Vitro* conservation. In *Proceedings of the International Conference on Plant, Marine and Environmental Sciences (PMES-2015)* (pp. 1–2). Kuala Lumpur, Malaysia.

Gambino, G., Ruffa, P., Vallania, R., & Gribaudo, I. (2007). Somatic embryogenesis from whole flowers, anthers, and ovaries of grapevine (*Vitis* spp.). *Plant Cell, Tissue and Organ Culture, 90,* 79–83. https://doi.org/10.1007/s11240-007-9251-5

Germana, M. A., Chiancone, B., Padoan, D., Barany, I., Risueno, M. C., & Testillano, P. S. (2011). First stages of microspore reprogramming to embryogenesis through anther culture in *Prunus armeniaca* L. *Environmental and Experimental Botany, 71,* 152–157. https://doi.org/10.1016/j.envexpbot.2010.11.001

Horvath, A., Balsemin, E., Barbot, J. C., Christmann, H., Manzano, G., Reynet, P., & Laigret, F. (2011). Phenotypic variability and genetic structure in plum (*Prunus domestica* L.), cherry plum (*P. cerasifera* Ehrh.), and sloe (*P. spinosa* L.). *Scientia Horticulturae, 129,* 283–293. https://doi.org/10.1016/j.scienta.2011.03.036

Ikram, N. K., Zhan, X., Pan, X. W., King, B. C., Simonsen, H. T., & Christensen, S. B. (2017). Genetic transformation of *Artemisia annua*. *Plant Biotechnology Reports, 11*(1), 1-13. https://doi.org/10.1007/s11816-016-0425-5

Jonard, R. (1986). Micrografting and its applications to tree improvement. In Y. P. S. Bajaj (Ed.), *Biotechnology in Agriculture and Forestry: Trees I* (pp. 31–48). Springer. https://doi.org/10.1007/978-3-642-71050-1_2

Kathiresan, K., & Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems. *Advances in Marine Biology, 40,* 81-251. https://doi.org/10.1016/S0065-2881(01)40003-4

Khan, S., Mirza, K. J., & Abdin, M. Z. (2010). Development of RAPD markers for authentication of medicinal plant *Cuscuta reflexa*. *Eurasia Journal of Biosciences, 4(1),* 1–7.

Khan, S., Mirza, K. J., Tyagi, M. R., & Abdin, M. Z. (2011). Development of RAPD markers for authentication of *Ruta graveolens* L. and its adulterant. *Medicinal and Aromatic Plants Science and Biotechnology, 5(1),* 58–61.

Kikkert, J. R., Striem, M. J., Vidal, J. R., Wallace, P. G., Barnard, J., & Reisch, B. I. (2005). Long-term study of somatic embryogenesis from anthers and ovaries of 12 grapevine (*Vitis* sp.) genotypes. *In Vitro Cellular & Developmental Biology-Plant, 41,* 232–239. https://doi.org/10.1079/IVP2005648

Kiran, U., Khan, S., Mirza, K. J., Ram, M., & Abdin, M. Z. (2010). SCAR markers: A potential tool for authentication of herbal drugs. *Fitoterapia, 81,* 969–976. https://doi.org/10.1016/j.fitote.2010.07.016

Kumar, V., Murthy, K. N., Bhamid, S., Sudha, C. G., & Ravishankar, G. A. (2005). Genetically modified hairy roots of *Withania somnifera* Dunal: A potent source of rejuvenating principles. *Rejuvenation Research, 8*, 37–45. https://doi.org/10.1089/rej.2005.8.37

Kundu, S., Salma, U., Ali, M. N., Hazra, A. K., & Mandal, N. (2018). Development of transgenic hairy roots and augmentation of secondary metabolites by precursor feeding in Sphagneticola calendulacea (L.) Pruski. Industrial Crops and Products, 121, 206-215.

Liu, W. X., Liu, W. H., Wu, J., Gao, A. N., & Li, L. H. (2010). Analysis of genetic diversity in natural populations of *Psathyrostachys huashanica* Keng using microsatellite (SSR) markers. *Agricultural Sciences in China, 9(4),* 463–471. https://doi.org/10.1016/S1671-2927(09)60137-8

McComb, J., Bennett, I., & Tonkin, C. (1996). *In Vitro* propagation of *Eucalyptus* species. In A. Taki & R. Williams (Eds.), *Tissue Culture of Australian Plants* (pp. 112–156). University of New England: Armidale, NSW, Australia.

Mridula, M. R., & Nair, A. S. (2018). Rapid micropropagation of *Wrightia tinctoria* (ROXB.) R. Br.: A medicinal tree. *International Journal of Botany Studies, 3,* 126–131.

Murch, S. J., Ragone, D., Shi, W. L., Alan, A. R., & Saxena, P. K. (2007). *In Vitro* conservation and sustained production of breadfruit (*Artocarpus altilis*, Moraceae): Modern technologies for a traditional tropical crop. In S. Jain & H. Hagman (Eds.), *Protocols for Micropropagation of Woody Trees and Fruits* (pp. 279–288). Springer. https://doi.org/10.1007/978-1-4020-6352-7_26

Nishihara, M., Habu, Y., Tsubokura, Y., Morita, Y., Matsuda, T., & Tanaka, Y. (2020). Applications of CRISPR/Cas9 in orchid biology. *Frontiers in Plant Science, 11,* 261. https://doi.org/10.3389/fpls.2020.00261

Onay, A., Pirinç, V., Işıkalan, C., Adıyaman, F., Tilkat, E., & Başaran, D. (2003). *In vivo* and *In Vitro* micrografting of pistachio (*Pistacia vera* L.) cv. "Siirt." *Turkish Journal of Biology, 27*, 95–100.

Panis, B., Nagel, M., & Van den Houwe, I. (2020). Challenges and prospects for the conservation of crop genetic resources in field genebanks, *In Vitro* collections, and/or in liquid nitrogen. *Plants, 9,* 1634. https://doi.org/10.3390/plants9121634

Perez, J., Araya-Valverde, E., Garro, G., & Abdelnour-Esquivel, A. (2017). Analysis of stress indicators during cryopreservation of seeds of landrace maize (*Zea mays*). *CryoLetters, 38,* 445–454.

Popova, E. V., Shukla, M. R., McIntosh, T., & Saxena, P. K. (2015). *In Vitro* and cryobiotechnology approaches to safeguard *Lupinus rivularis* Douglas ex Lindl., an endangered plant in Canada. *Agronomy, 11,* 37. https://doi.org/10.3390/agronomy11010037

Popova, E., Shukla, M. R., Kim, H. H., & Saxena, P. K. (2015). Plant cryopreservation for biotechnology and breeding. In J. Al-Khayri, S. Jain, & D. Johnson (Eds.), *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools* (pp. 63–93). Springer. https://doi.org/10.1007/978-3-319-22521-0_3

Prado, M. J., Grueiro, M. P., Gonzalez, M. V., Testillano, P. S., Dominguez, C., Lopez, M., & Rey, M. (2010). Efficient plant regeneration through somatic embryogenesis from anthers and ovaries of six autochthonous grapevine cultivars from Galicia (Spain). *Scientia Horticulturae, 125,* 342–352. https://doi.org/10.1016/j.scienta.2010.04.007

Rathwell, R., Popova, E., Shukla, M. R., & Saxena, P. K. (2016). Development of cryopreservation methods for cherry birch (*Betula lenta* L.), an endangered tree species in Canada. *Canadian Journal of Forest Research, 46,* 1284–1292. https://doi.org/10.1139/cjfr-2016-0145

Reed, B. M. (2017). Plant cryopreservation: A continuing requirement for food and ecosystem security. *In Vitro Cellular & Developmental Biology-Plant, 53,* 285–288. https://doi.org/10.1007/s11627-017-9829-5

Reshi, N. A., Sudarshana, M., & Girish, H. (2018). *In Vitro* micropropagation of *Rhinacanthus nasutus* (L.) Kurz. *International Journal of Biodiversity and Conservation, 10,* 357–364. https://doi.org/10.5897/IJBC2018.1239

Rout, G. R., Senapati, S. K., Aparajita, S., & Palai, S. K. (2009). Genetic analysis of *Rauvolfia serpentina* using molecular markers. *Plant Cell, Tissue and Organ Culture, 99*(1), 33-40. https://doi.org/10.1007/s11240-009-9576-8

Sáez, P. L., Bravo, L. A., Latsague, M. I., Sánchez, M. E., & Ríos, D. G. (2012). Increased light intensity during *In Vitro* culture improves water loss control and photosynthetic performance of *Castanea sativa* grown in ventilated vessels. *Scientia Horticulturae, 138,* 7–16. https://doi.org/10.1016/j.scienta.2012.02.006

Salama, A., Popova, E., Jones, M. P., Shukla, M. R., Fisk, N. S., & Saxena, P. K. (2018). Cryopreservation of the critically endangered golden paintbrush (*Castilleja levisecta* Greenm.): From nature to cryobank to nature. *In Vitro Cellular & Developmental Biology-Plant, 54,* 69–78. https://doi.org/10.1007/s11627-018-9896-6

Sharma, N. (2001). *In Vitro* conservation of *Gentiana kurroo* Royle: An indigenous threatened medicinal plant. *Indian Journal of Plant Genetic Resources, 14,* 99–100.

Sharma, R., Kumar, V., & Rameshwaran, M. (2015). Conservation of *Taxus baccata* using biotechnological tools. *Forests, 6*(12), 4654-4670. https://doi.org/10.3390/f6124392

Singh, B., Sharma, S., Rani, G., Hallan, V., Zaidi, A. A., Virk, G. S., & Nagpal, A. (2008). *In Vitro* micrografting for production of Indian citrus ring spot virus (ICRSV)-free plants of kinnow mandarin (*Citrus nobilis* Lour × *C. deliciosa* Tenora). *Plant Biotechnology Reports, 2*, 137–143. https://doi.org/10.1007/s11816-008-0054-y

Thomas, T. D., Jebadhas, A. W., & Nair, M. S. (2017). Somatic embryogenesis in *Syzygium travancoricum*. *Plant Cell Reports, 36*(7), 1003-1011. https://doi.org/10.1007/s00299-017-2133-6

Usman, I. S., & Abdulmalik, M. (2010). Cryopreservation of embryonic axes of maize (*Zea mays* L.) by vitrification protocol. *African Journal of Biotechnology, 9*, 8955–8957. https://doi.org/10.5897/AJB2010.000-3403

Wang, S., Yang, W., & Shen, H. (2011). Genetic diversity in *Apium graveolens* and related species revealed by SRAP and SSR markers. *Scientia Horticulturae, 129*, 1–8. https://doi.org/10.1016/j.scienta.2011.02.014

Whiteley, S. E., Bunn, E., Menon, A., Mancera, R. L., & Turner, S. R. (2016). Ex situ conservation of the endangered species *Androcalva perlaria* (Malvaceae) by micropropagation and cryopreservation. *Plant Cell, Tissue and Organ Culture, 125*, 341–352. https://doi.org/10.1007/s11240-016-0967-5

Zuzarte, M. R., Dinisa, A. M., Cavaleiro, C., Salgueiro, L. R., & Canhoto, J. M. (2010). Trichomes, essential oils, and *In Vitro* propagation of *Lavandula pedunculata* (Lamiaceae). *Industrial Crops and Products, 32*, 580–587. https://doi.org/10.1016/j.indcrop.2010.07.011