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Sustainability of *In Vitro* Genebanks and Cryogenebanks

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PGRs are essential components of food, nutrition and livelihood security and in the last few decades significant improvements have been made for better and safe conservation in national and international genebanks. There are several species, which cannot be conserved in conventional seed genebanks and needs alternate strategies. Among different strategies *in vitro* and cryogenebanks are the only available approaches for safe conservation of difficult species for short- to medium-term and for long-term respectively. *In vitro* and cryoconservation is in practice for several years, slow growth conservation and cryoconservation protocols are developed in diverse species and many species are successfully and safely conserved in *in vitro* and cryogenbanks in many countries globally. This paper reviews the priority species and future prospects of *in vitro* and cryogenebanks.

Introduction

Plant genetic resources (PGR) are fundamental for life on earth. Globally plant genetic heritage is at greater erosion due to globalization, industrialization, degradation of natural habitats, climate change, replacement of traditional varieties with modern cultivars and hybrids, agriculture intensification, overexploitation from natural habitats, population increase, land degradation etc (Gowthami *et al.,* 2021). Worldwide 17.01 % of the world's vascular plants (3,42,953) are under varying degrees of threat and in the view of 'sixth mass extinction', there is a global alarm for greater need for immediate efforts for conservation of plant diversity for the sustainable future of the planet. Conservation of PGRs in their natural habitat (*in situ*) is the first and primary approach being followed from the time immemorial, but due to vulnerability to natural hazards, a safety backup conservation approaches is required. Then emerged a concept of *ex situ* conservation (conservation away from the natural habitat) as botanical gardens, herbal gardens, arboreta, sacred groves and gene banks. Approximately 92% of angiosperms (3, 30,000) produce orthodox seeds (desiccation-tolerant), which tolerate drying to low moisture content (≤ 5%) without a significant loss of viability. These seeds can be conserved conventionally in seed genebanks, that remains viable for 25-50 years under refrigerated conditions (5-10 ^{o}C)) and can survive ranged from 100–200 years in the deep freezer (-20 ^{o}C). However, conservation of all the seed species are not possible in seed genebanks and is not suitable for all the seed species. Germplasm collections were initiated in 1920 mainly for breeding purpose and during 1960s & 1970s, major emphasis was given for conservation of major food crops that produce orthodox seeds and research was biased towards these crops, which eventually led to the establishment of present day, nearly 1,750 seed genebanks globally. However, conservation in seed genebanks is not feasible for many plants/crops, which producing difficult to conserve-species (Fig 1A). These species are normally conserved as living collections in field genebanks, but it requires large area, labour intensive, high maintenance cost, easily prone to natural hazards, pest/pathogen attacks etc. The alternative safe and sustainable approaches for the conservation of these difficult-to-conserve species is *in vitro* conservation (short- to medium term) and cryo-conservation (long-term conservation).

In Vitro and Cryogenebank

In vitro genebank represents the conservation of plant genetic resources in the form of *in vitro* cultures under controlled conditions. The potentiality of *in vitro* techniques for safe conservation of difficult-to-conserve species was emphasized in the early 1970s, later in 1980s, International Board for Plant Genetic Resources (IBPGR) formed a specialists working group to evaluate the critical aspects of *in vitro* plant conservation. During 1987-89, the technical and logistical aspects of establishing and running an *in vitro* active genebank using cassava as a model were assessed. Later in 1997, IBPGR status report on *in vitro* conservation techniques was developed and in 2004 technical guidelines for the management

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of field and *in vitro* collections was developed. In 2014, with an objective of conservation of PGR with recognized and appropriate standards based on current and available technological and scientific knowledge, genebank standards for PGRFA maintained *in vitro* conservation was developed. In *in vitro* genebanks, PGRs can be conserved for short- to medium-term period (several months to a few years) under normal growth conditions (standard culture room conditions) or subjected to growth-limiting conditions using one or more slow growth conservation strategies *viz.*, low temperature storage, low light storage, use of minimal growth media, use of osmotica in the medium, reduced oxygen concentration, type of culture tube enclosures and culture vessels, desiccation and encapsulation, induction of storage organs etc. (*in vitro* active genebank) and for long-term period under suspended growth (cryoconserved) in liquid nitrogen (*in vitro* base genebank) (Agrawal *et al*., 2019; Sharma *et al*., 2020) (Fig. 1B). A huge amount of literature has been published on the application of *in vitro* genebank for sustainable conservation of PGR. Protocols for *in vitro* conservation were developed in a large number of crops (> 1000 species) justifies the successful maintenance of *in vitro* genebanks from several years.

Cryogenebank represents the conservation of PGRs at ultra-low temperature in liquid nitrogen (LN) either in liquid phase (-196 °C) or in vapor phase (-150 to -180 °C). The first scientific report on survival of plant tissues after exposure to liquid nitrogen dates back to 1956, since then major evolutionary developments in the field of plant cryobiology was reported particularly with the evolution of different techniques *viz.*, from classical cryopreservation techniques (slow cooling) to vitrification based techniques (pregrowth, pregrowth-dessication, vitrification, droplet vitrification, encapsulation-dehydration, encapsulation-

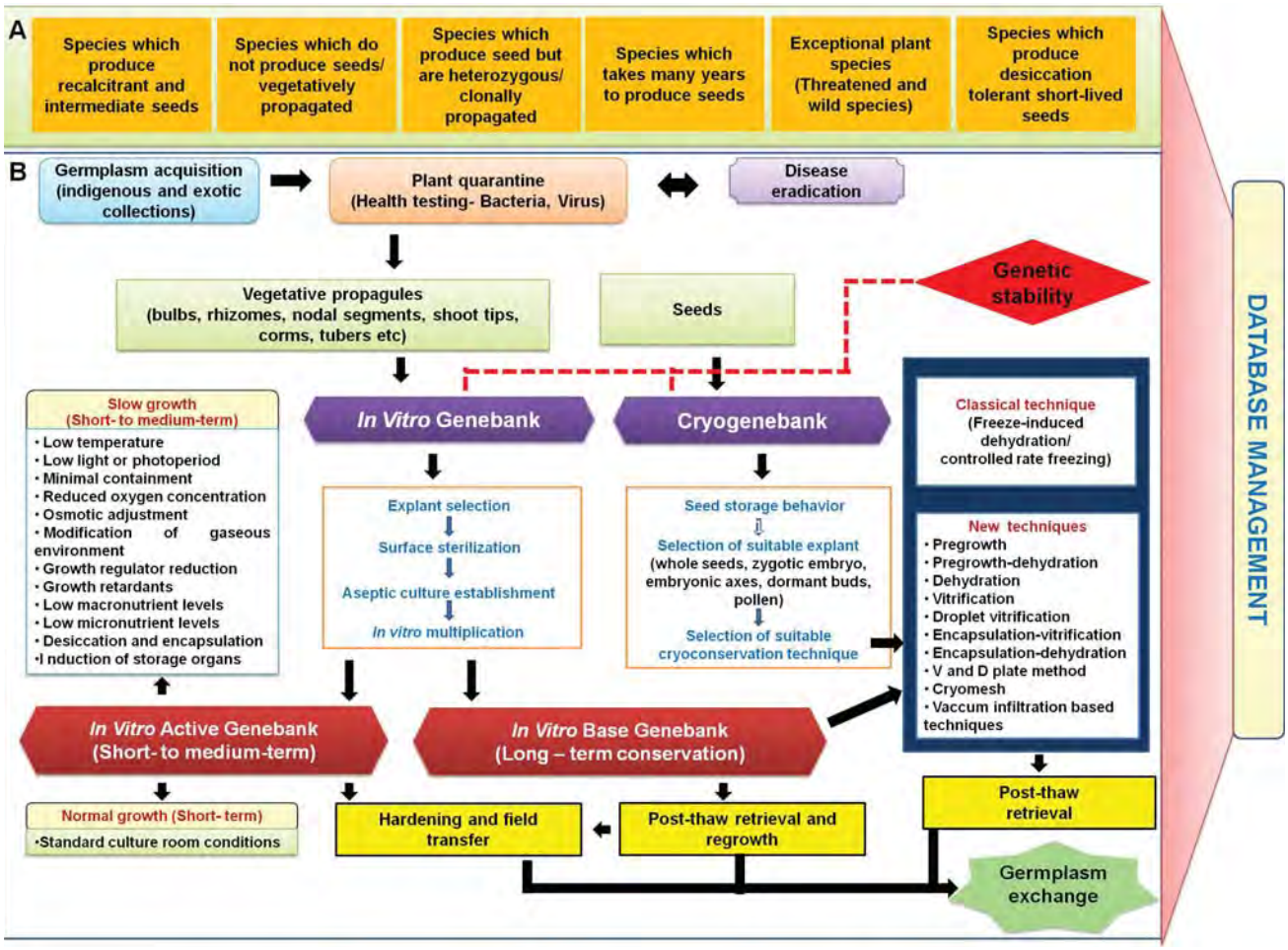

Fig. 1. *In vitro* **genebank and cryogenebank (A) Priority species categories for *in vitro* and cryoconservation (B) Schematic representation of different activities in *in vitro* and cryogenebanks**

vitrification, V and D cryoplates) and recently developed techniques of cryomesh and vacuum infiltration. So far, cryoconservation is applied to diverse groups of plant tissues *viz.*, whole seeds, zygotic embryos, embryonic axes, dormant buds, shoot tips, hairy roots, shoot meristems, axillary buds, nodal segments, bulbils, somatic embryos, embryogenic cell suspensions etc.). From the six decades after its birth, cryoconservation protocols were developed in diverse species for long-term conservation (Agrawal *et al*., 2019; Sharma *et al*., 2020). *In vitro* and cryogenebanks has several advantages than the field genebanks in conservation of difficult-to-conserve species and Fig. 2 provides their SWOT analysis.

In vitro and Cryogenebank of ICAR-NBPGR: 36 Years Successful Journey

In vitro conservation and cryo-conservation of difficult-to conserve species was initiated at ICAR-NBPGR, New Delhi in 1986 and led to the foundation for National Facility for Plant Tissue Culture Repository-NFPTCR (presently Tissue Culture and Cryopreservation Unit), a state-of -the- art -facility in the area of plant tissue culture *(in vitro* genebank) and cryo-conservation (cryogenebank) representing the unique multi crop repository (Fig. 3) to conserve clonally propagated plant species and recalcitrant seed species with funding from the Department of Biotechnology (DBT), Government of

Fig. 2. Strength, weakness, opportunity and threat (SWOT) analysis of *in vitro* and cryogenebanks

Fig. 3. *In Vitro* and Cryogenebank of ICAR-NBPGR, New Delhi. (A) *In Vitro* Active Genebank (B) *In Vitro* Base Genebank and (C) Cryogenebank

India. Currently, a total of 1,943 accessions belonging to >59 genera and >149 species belonging to six crop groups *viz.,* (a) Tropical fruits (447), (b) Temperate and minor tropical fruits (382), (c) Tuber crops (527), (d) Bulbous and ornamental crops (173), (e) Medicinal & aromatic plants (186) and (f) Spices and industrial crops (228) are maintained either as *in vitro* cultures under normal growth (25 °C temperature, 16/8 h photoperiod regime) in the IVAG (Figure 3A) with a subculture period of 1 to 24 months, depending on the species/genotype. A total of 297 accessions of *Allium* sp., *Bacopa monnieri, Colocasia esculenta, Dioscorea* sp., *Ensete* sp., *Fragaria* sp., *Garcinia indica, Gentiana kurroo, Musa* sp., Rubus hybrid and *Vaccinium ovatum* were cryoconserved in IVBG (liquid phase of LN) using different cryo-conservation techniques. In addition, a total of 12,274 accessions belonging to more than 820 species have been cryoconserved as seeds, zygotic embryos, embryonic axes, dormant buds and pollen and 2,194 genomic resources (DNA) in Cryogenebank (vapor phase of LN) (Agrawal *et al*., 2022). With the application of efficient slow growth conservation and cryoconservation protocols, ICAR-NBPGR *In vitro* and Cryogenebank was able to conserve the germplasm without any loss during the recent incidence of COVID-19 pandemic.

Future Prospective and Action Points

- It is estimated that at least 36% of red list species are most likely to produce recalcitrant seeds. Major emphasis should be given to the protocol development for conservation of these species in *in vitro* and Cryogenebank.
- Development of efficient protocols for vegetatively propagated and non-orthodox seeded crops, with emphasis on under-utilized crop species, rare/ endangered plants and their wild relatives.
- Need to develop generic protocols for cryo-conservation of diverse PGRs.
- There is a need for National and International collaborations, and networks for delineating the constraints in *in vitro* and cryo-conservation of difficult to conserve species.
- Database of *in vitro* and Cryogenebanks across the world needs to be developed for efficient knowledge sharing and material transfer.
- *In vitro* genebank and Cryogenebanks receives less importance in comparison to the seed genebanks with respect to funding. Hence there is a need for government support for establishment of *in vitro* and Cryogenebanks for conservation of difficult to conserve species.
- Emphasis should be given for safety duplication of *in vitro* and cryoconserved germplasm.
- Development of low-cost slow growth conservation strategies for germplasm conserved in the IVAG.
- Automation in *in vitro* and Cryogenebank with robotic technology.
- Capacity building through trainings on *in vitro*/ cryoconservation at national and international levels.

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