ISSN: 3049-0677 (Online)

PhytoTalks

October 2025; Volume 2: Issue 3: 478-483
https://doi.org/10.21276/pt.2025.v2.i3.5

Safavid e-publishers

Research Article

OPEN ACCESS

**Article History**
Received: 11.08.2025
Revised: 23.09.2025
Accepted: 29.09.2025
Online
Version: 1

**Additional Information**
**Peer review**: The publisher expresses gratitude to the anonymous reviewers and sectional editors for their invaluable contributions during the peer review process.

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https://phytotalks.com/journal/index.php/PT/open_acess_policy

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**Cite this article:** Yadav S, Priya K, Kandari P, Thakur A. *In vitro* minimal growth and pollen cryostorage as an approach to restoration of threatened forest tree species, *Oroxylum indicum* (L.) Benth. ex Kurz. *PhytoTalks*. 2025; 2(3): 478-483.

# *In vitro* minimal growth and pollen cryostorage as an approach to restoration of threatened forest tree species, *Oroxylum indicum* (L.) Benth. ex Kurz

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## Abstract

Planting tree species is a sustainable approach to achieve landscape restoration but maintaining the diverse native flora of the region being restored is also very crucial to guarantee balanced representation by all species. Many tree species are facing threat to existence in their natural habitats owing to land degradation, competition and anthropological activities. *Oroxylum indicum* (Bignoniaceae), is one of those species which is categorized as threatened due to overexploitation combined with poor seed set, rare pollinators and low seed viability. Hence the plant needs to be conserved through appropriate strategies and in vitro conservation of germplasm in the form of plant cultures and pollen grains offers a suitable alternative. The research presented here describes the effect of culture medium modifications on medium term storage of cultures in *O. indicum* as well as methods for cryostorage of pollens from the species. For minimal growth, modifications in the form of decreased macronutrients (1/2 and 1/4), increased sucrose (5%) and use of mannitol (1%) in the medium were done. The shoot length and leaf number decreased during minimal growth treatments with respect to control and could be proliferated again in regrowth medium comprising of Murashige and Skoog's growth medium along with kinetin and glutamine in 0.5 mg $L^{-1}$and 25 mg $L^{-1}$ concentration, respectively. For pollen conservation, initial moisture of the collected pollens was determined as 28% and viability was almost 100% when germinated on in vitro medium containing boric acid, chlorides of calcium and potassium, magnesium sulfate, and sucrose. The pollens could be stored for 3-6 months in -196°C temperature and remain 55% and 20% viable after three and six months, respectively. The findings will help devise methods to proper management of germplasm resources in this species through *ex situ* conservation so that the conserved plants can be reintroduced into degraded natural habitat of the species. Since the species has wide uses and has been utilized by traditional Indian communities, conserving its population would ultimately also benefit livelihoods of people associated with it.

**Keywords:** *In vitro* conservation, *Oroxylum indicum*, plant cultures, pollen grains, restoration.

## 1. Introduction

Forests are an integral part of humankind's survival and development. These are rich in biodiversity and provide multifarious economic and ecological benefits. However, the world is witnessing a distressing depletion of forest landscapes due to factors like uncontrolled human interference in the form of illegal felling of trees, mismanaged land use changes, extreme climate change and so on. This alarming trend intensifies critical need for the conservation of forest flora and fauna equally[1]. The aim of landscape restoration is achieved in true sense when diverse flora of restored region is represented in a balanced way[2]. Among the many imperiled forest tree species is *Oroxylum indicum* ((L.) Benth. ex Kurz, commonly referred to as the Indian trumpet tree or "Shyonaka" in Ayurveda. Native to India, Bangladesh, and Sri Lanka, this majestic deciduous tree holds immense ecological significance due to its association with ancient medicinal traditions and its vital role as a habitat and food source for a diverse array of wildlife species[3].

Unfortunately, *O. indicum* has faced a precipitous decline in population across its natural range, particularly in South and Southeast Asia, primarily due to factors such as habitat degradation and anthropological interference[4]. Conventional conservation methods, including habitat preservation and *ex situ* conservation in botanical gardens, have yielded sporadic success. These approaches often present challenges such as substantial space requirements, the risk of inbreeding, and exposure to suboptimal nutrition and pathogens[5]. As an alternative, biotechnological interventions such as maintenance of plant cultures under *in vitro* minimal growth and pollen cryostorage have emerged as novel techniques of conservation. *In vitro* minimal growth is a technique that allows for the laboratory-based storage of plant species for short to medium term. During *in vitro* conservation of plants, cultures are grown in controlled light and temperature conditions, and the basic aim is to extend the subculture durations through culture medium modifications, and changes in temperature or light conditions[6]. Simultaneously, pollen cryostorage is a method of long-term preserving viable pollens of plant species in ultra-low temperatures while ensuring viability or longevity. Such approaches of *in vitro* conservation that include conservation of germplasm in form of pollens or tissue cultures are a promising way to ensure restoration of threatened plant species, such as *O. indicum*[7]. Additionally, these methods also safeguard the genetic diversity of any species, thereby ensuring that future restoration endeavors can reintroduce genetically diverse individuals into their native environments.

Earlier studies have explored *in vitro* conservation of *O. indicum* through tissue culture techniques, utilizing various types of growth media and explant propagules[8, 9]. Pollen storage, both short-term and long-term via cryostorage, has also been investigated by Harsha *et al.*[10]. The current study holds particular significance for germplasm conservation as it covers simultaneous investigations into medium-term storage using modified culture growth conditions and long-term storage under cryo-conditions, while also including pollen moisture analysis and optimization of growth media for determining viable pollen germination. The findings will help devise methods to manage proper germplasm resources in this species through *ex situ* conservation

## 2. Materials and Methods

### *2.1. In vitro minimal growth experiments*

For *in vitro* minimal growth experiments, pre-established shoot cultures were used, and these were maintained through regular subcultures in multiplication medium comprising of Murashige and Skoog's (MS) medium[11] supplemented with kinetin

and glutamine in 0.5 mg $L^{-1}$ and 25 mg $L^{-1}$ concentration, respectively. Different *in vitro* treatments for slow growth of cultures were experimented as follows: *T1*and *T2*: MS medium with half and one-fourth the concentration of macroelements, respectively and supplemented with kinetin (0.5mg $L^{-1}$), *T3*: Basal MS medium with increased sucrose (5% w/v) and kinetin (0.5 mg $L^{-1}$), *T4*: Basal MS medium with mannitol (1% w/v) and kinetin (0.5 mg $L^{-1}$), and *T5*: Basal MS medium with mannitol (1% w/v), reduced sucrose (1% w/v) and kinetin (0.5 mg $L^{-1}$). The shoot multiplication medium, termed here as 'SMM' was taken as control. Data of slow growth was taken at four weeks interval and viability of cultures was checked by regrowth in shoot multiplication medium. For growth, cultures were kept in culture room conditions with temperature maintained at 25±2 °C, relative humidity at 55±5% and a photoperiod of 16/8 h (light/dark). Light was supplied by cool-white, fluorescent tubes (Philips) at an intensity of 35 μmoles/$m^2$/s.

### *2.2. Pollen cryostorage experiments*

The experimental material consisted of flower buds and pollens collection was followed the anthers dehisced. For initial moisture determination, freshly weighed pollens were oven dried in aluminum foil cups for 17 hours at 103°C. Finally, the dry weight was measured, and moisture content was estimated through following formula:

Moisture content = [(Initial weight before drying-Final weight after drying)/ (Initial weight before drying-weight of empty aluminum foil cup)] * 100.

Before storing the pollen grains the moisture levels between 11-13% are considered suitable and this can be achieved through desiccation. To check the pollen viability, *in vitro* germination was performed in different growth media. Brewbaker & Kwack's medium[12] was termed as *Pollen germination medium* (PGM)1 and a 100 mL of this medium contained 0.01% boric acid, 0.03% calcium nitrate, 0.02% magnesium sulfate, 0.01% potassium nitrate, 5 to 20% sucrose and 1% agar. The other medium termed as *PGM 2* contained 0.01% boric acid, 0.04% calcium chloride, 8% sucrose, 1% each agar and gelatin , and *PGM 3* medium contained 0.01% boric acid, 0.07% calcium chloride, 0.03% potassium chloride, 0.02% magnesium sulfate, 10%sucrose, and 1.5% agar. Pollen grains were dusted over the thin layer of solidified medium placed over a glass slide and incubated at 26-27°C for 12-24 hours. Pollen tube growth is observed microscopically and if the length is greater than or equal to diameter of the pollen then the germination is considered 100%. All the results were imaged through a digital camera during microscopy.

Properly desiccated pollen grains were stored in pre chilled cryovials, placed carefully in cryo-canisters and immersed in liquid nitrogen (LN) Dewar vessel for cryostorage. Samples were checked for viability after three and six months of storage by taking out the tubes from LN, instantly thawing them in water bath at 37 °C and then plated on to standardized growth medium for viability check in the form of pollen tube emergence.

## 3. Results and Discussion

The *in vitro* raised shoots were cultured in five different treatments in order to check the minimal growth and enhance subculture duration, a criteria important for medium term storage. Average shoot length and leaf number were recorded in each treatment to ascertain best culture medium inducing minimal growth. Medium combination with half the macronutrient concentration and 0.5 mg $L^{-1}$ kientin was most suitable for slow growth with lowest shoot length of 1.1 cm after 3 months of storage. However, same level of significance between T1 and T2 was observed. Shoot length was higher in all other combinations (T3, T4, T5) than T1 after 3 months of storage. This result clearly indicated the effect of decreasing the macroelements concentration in culture medium when compared with modifications like addition of osmotic agents (sucrose and mannitol). Lower macroelements could yield better outcomes in case of slow development of plants since it inhibits plant metabolism and shoot elongation[13]. High concentrations of osmotic regulators such as sorbitol, mannitol and sucrose may create osmotic imbalance or stress which although slows the rate of growth but can be detrimental to plant. However, variations can be seen with respect to type of osmotic regulator and concentration, the target plant species, and the physical growth conditions which may be the

reason why the osmotic regulators gave poorer results[14]. Novero *et al.*[15] observed that addition of osmotic regulators like sucrose and sorbitol increased the morphological growth parameters of Sago Palm. Overall, the treatments in present study had lower values of shoot length with respect to control which highlighted the positive impact of various growth reducing parameters used for inducing slow growth in *O. indicum*. Another parameter that was considered in optimizing best suitable medium for minimal growth storage of *O. indicum* cultures was leaf number. The results show that macroelements concentration was an important parameter which when modified to ½ the original concentration produced minimum leaf number (1.8) during the storage period of 3 months. The results obtained with treatment 1 were at par with those obtained for treatment 2 and treatment where 1% sucrose and mannitol each were incorporated in the medium (Figure 1b). It was therefore interpreted that a reduction in macroelements concentration and sucrose along with incorporation of osmotic regulators like mannitol in the medium had similar effects on leaf number. This could be because additive effect of sucrose and mannitol may lead to alteration in the amount of water uptake and cell turgor pressure thus creating osmotic stress which may impact cell division and expansion negatively, thereby hindering leaf development[16]. Figure 1a and 1b describe the effect of modifications in medium on mean shoot length and leaf number in *O. indicum* cultures after 3 months storage.

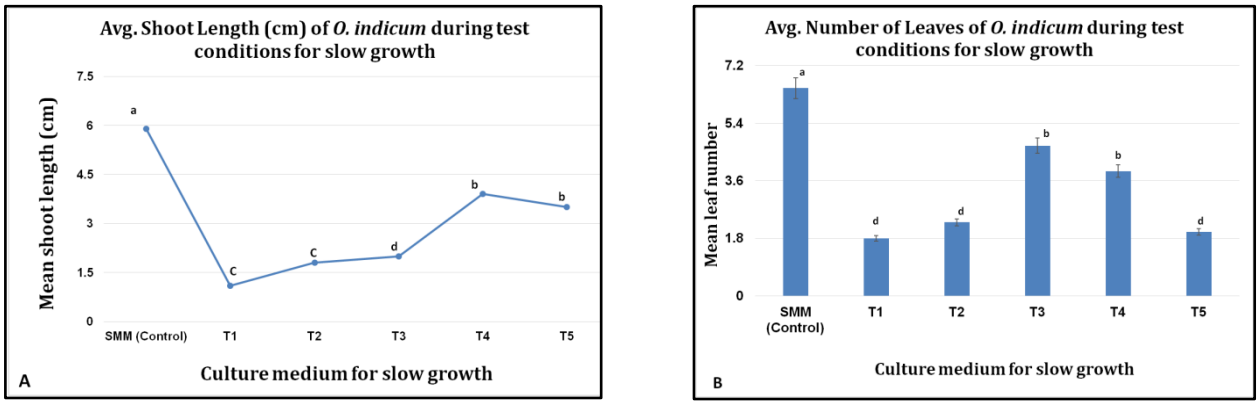


**Figure 1a, b:** Effect of storage conditions on mean shoot length and leaf number in O. indicum cultures. MS medium with ½ concentrations of macronutrients (T1) showed lowest mean shoot length (1a) and lowest mean leaf number (1b) after 3 months of storage.

Reduced macroelements concentration may also limit the availability of nutrients and organic compounds that are required for plant growth and leaf development[14]. Sucrose and mannitol when used individually, did not give favorable results indicating that osmotic regulators alone may not be sufficient to considerably affect plant metabolism and growth rate in *O. indicum* shoot cultures. As a result, combining osmotic regulators with lower macroelements concentrations may have a synergistic effect on reducing plant development and extending the subculture period[17].Thus, in the present study, shoot cultures were successfully stored under minimal growth conditions for 3 months period and grew normally when subcultured in regular shoot multiplication medium (SMM).

In the case of pollen cryostorage, the prerequisite is to first determine the initial moisture content of pollens and hence oven-drying and desiccation method was used to decipher moisture content. The average moisture content of the pollens of *O. indicum* was found to be 28%. The initial moisture content reported in the study by Harsha *et al.*[10] is 34%. Pollen viability and storage duration are affected by moisture content and low moisture content is generally preferable for conserving pollen as it prevents ice crystal formation when pollens are subjected to ultra-low temperature. Factors like plant type, storage

temperature, and drying process also affect the result determination. In the current study, the moisture content was brought to 11-13% through desiccation before storing the pollens in liquid nitrogen. Germination, *in vitro*, was best obtained as 98.26% in medium *PGM 3*which had chloride salts of calcium and potassium in higher concentration and 1.5% agar when compared to other two media types used (Figure 2). These results indicate that chloride salts of calcium and potassium were more suited for the growth of pollens of *O. indicum* than the standard B & K medium which contained calcium and potassium in the form of nitrates. The studies by Leduc *et al.*[18] in *Capsella bursa-pastoris* and in *Cajanus cajan* by Jayaprakash and Sarla[19] also showed better germination in pollens in other media types rather than in B & K medium. The data obtained indicates that the pollen could be stored effectively for 3 months with 55% viability and at the end of six months storage the viability decreased up to 20% which differed from the studies by Harsha *et al.*[10]. This may be due to different storage conditions and protocols followed. Pollen germination characteristics of pollens held in liquid nitrogen are generally reduced upon retrieval[20]. The findings from present study could help devise methods for proper management of germplasm resources in this species through *ex situ* conservation so that the conserved plants can be propagated and reintroduced into natural habitat of the species.

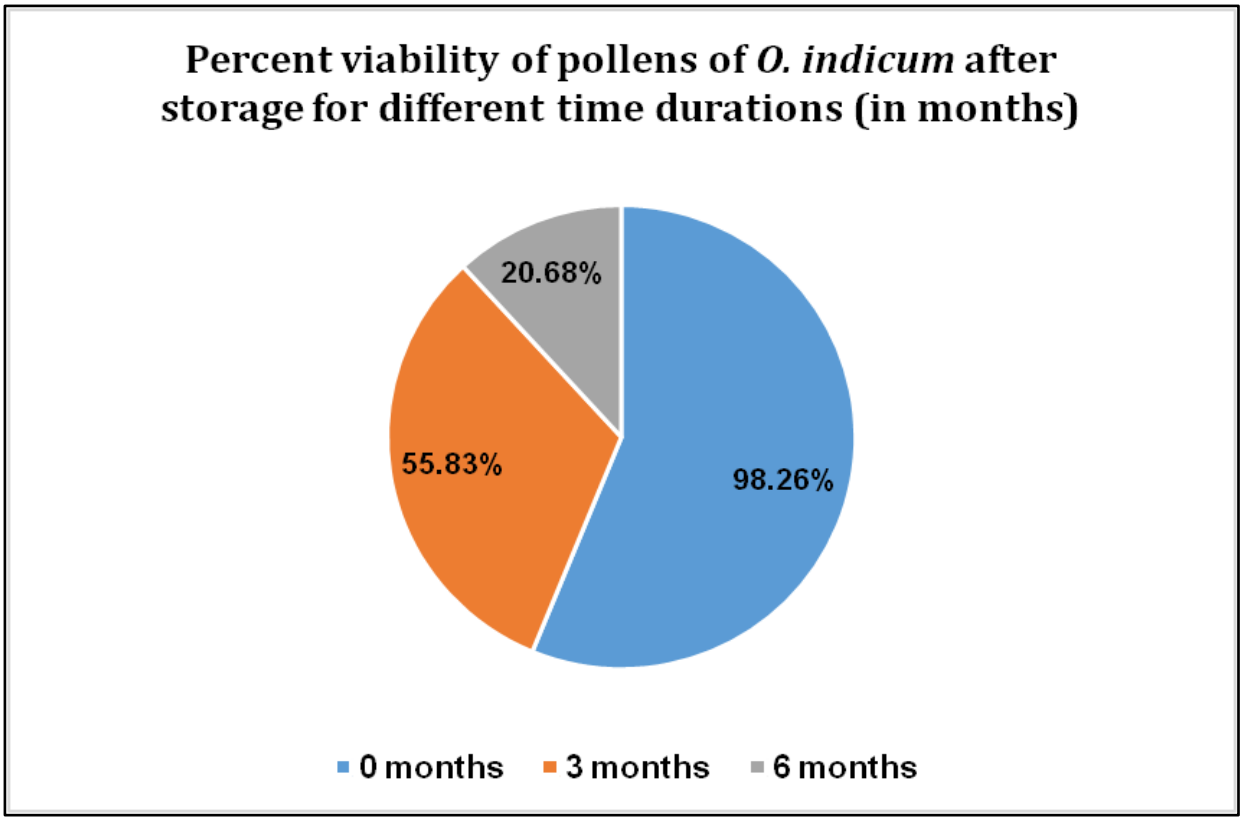


**Figure 2**: Pie chart showing viability of pollens in *O. indicum* in PGM 3 medium as observed after 0, 3, and 6 months of storage

## 4. Conclusion

The present study indicates that *in vitro* techniques like minimal growth cultures and pollen cryopreservation are suitable alternatives available to conserve species. Here in this study, *in vitro* proliferating *O. indicum* shoots could be successfully maintained, without any intervening subculture step, for three months under normal growth room conditions. The stored shoots could be multiplied normally after storage thereby establishing the viability of cultures. Pollen grains of *O. indicum* were also stored in cryo conditions and these could be preserved for 3 and 6 months with 55.83% and 20.68% viability respectively. Conservation through these methods offers

advantage of maintaining disease free germplasm in a limited space and further refinements can be done to target storage for longer durations.

## Acknowledgments

This work was financially supported as research grant by National CAMPA Fund, Ministry of Environment, Forest & Climate Change, Government of India in the form of pilot project on 'National Program for Conservation and Development of Forest Genetic Resources & Establishment of Centre of Excellence on Forest Genetic Resources (CoE-FGR)'. The authors are thankful to the Director, Forest Research Institute, Dehradun for providing laboratory and field works facilities.

## Author Contributions

SY: Conceptualization and research design; data acquisition, analysis, and interpretation; KP, statistical analysis and manuscript preparation; PK: manuscript preparation, review and editing. AT: Manuscript review, editing, and final corrections. All authors have read and approved the final version of the manuscript.

## Data Availability Statement

The raw data underpinning this article's conclusions can be obtained from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## References

1. Ahmed MEAE. *In vitro* propagation for conservation and genetic fidelity of the near threatened *Dimocarpus longan* plant. *J Genet Eng Biotechnol.* 2022; 20: 130.https://doi.org/10.1186/s43141-022-00406-4
2. Jacobs DF, Dalgleish HJ, Nelson CD. A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction. *New Phytol.* 2013; 197(2): 378-393. https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.12020
3. Lopresti AL, Smith SJ, Majeed M, Drummond PD. Effects of an *Oroxylum indicum* Extract (Sabroxy®) on cognitive function in adults with self-reported mild cognitive impairment: a randomized, double-blind, placebo-controlled study. *Front Aging Neurosci.* 2021; 13: 728360. https://doi.org/10.3389/fnagi.2021.728360
4. Dalal NV, Rai VR. *In vitro* propagation of *Oroxylum indicum* Vent. a medicinally important forest tree. *J For Res.* 2004; 9(1): 61-65. https://link.springer.com/article/10.1007/s10310-003-0055-x
5. Kasso M, Balakrishnan M. *Ex situ* conservation of biodiversity with particular emphasis to Ethiopia. *ISRN Biodiversity.* 2013; 2013 (985037): 11. https://onlinelibrary.wiley.com/doi/10.1155/2013/985037
6. Chauhan R, Singh V, Quraishi A. In vitro conservation through slow-growth storage. In: Faisal M, Alatar A, eds. *Synthetic Seeds*. Cham: Springer; 2019:397-416. https://doi.org/10.1007/978-3-030-24631-0_19
7. Chaudhury R, Malik SK, Rajan S. An improved pollen collection and cryopreservation method for highly recalcitrant tropical fruit species of mango (*Mangifera indica* L.) and litchi (*Litchi chinensis* Sonn.). *Cryo Letters.* 2010; 31(3): 268-278. https://pubmed.ncbi.nlm.nih.gov/20919456/
8. Talari S, Rudroju S, Nanna RS. Conservation of an endangered medicinal forest tree *Oroxylum indicum* (L.) Kurz through *in vitro* micropropagation-a review. *European J Med Plants.* 2016; 17(2): 1-13.
9. Samatha T, Rama Swamy N. Conservation of an endangered medicinal forest tree species, *Oroxylum indicum* L. Kurz, through in vitro culture: a review. In: Khasim SM, Long C, Thammasiri K, Lutken H, eds. *Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation*. Singapore: Springer; 2020:769-783.
10. Harsha R, Rajeshwari D, Rajasekharan PE. Pollen storage studies in *Oroxylum indicum* (L.) Vent. In: International E-Conference on Advances and Future Outlook in Biotechnology and Crop Improvement for Sustainable Productivity; November 24-27, 2020; Bengaluru, Karnataka, India.
11. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant.* 1962; 15(3):473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
12. Brewbaker J.L., Kwack B.H. The essential role of calcium ion in pollen germination and pollen tube growth. *Amer J Bot.* 1963; 50(9): 859-865. https://doi.org/10.1002/j.1537-2197.1963.tb06564.x
13. Luz T.C.L.A., Cardoso L.D., Alves R.B.N., and Matsumoto, K. Effect of osmotic regulators on *in vitro* conservation of brazilian ginseng, potato and cassava germplasms. *Acta Hortic.* 2015; 1083: 519-525
14. George EF, Hall MA, De Klerk GJ. The components of plant tissue culture media II: organic additions, osmotic and pH effects, and support systems. In: George EF, Hall MA, De Klerk GJ, eds. *Plant Propagation by Tissue Culture. Vol 1. The Background.* Dordrecht: Springer; 2007:115-173.
15. Novero A.U., Delima A.G., Acaso J., and Baltores L.M. The influence of osmotic concentration of media on the growth of Sago Palm (*Metroxylon sagu* Rottb.) *in vitro*. *Aust J Crop Sci.* 2010; 4(6): 453–456.
16. Zivcak M, Brestic M, Sytar O. Osmotic adjustment and plant adaptation to drought stress. In: Hossain M, Wani S, Bhattacharjee S, Burritt D, Tran LS, eds. *Drought*

*Stress Tolerance in Plants. Vol 1*. Cham: Springer; 2016:105-143. doi:10.1007/978-3-319-28899-4_5

17. Muñoz M, Diaz O, Reinún W, Winkler AJ, Quevedo R. Slow growth *in vitro* culture for conservation of Chilotanum potato germplasm. *Chil J Agric Res*, 2019; 79(1):26–35.
18. Leduc N, Monnier M, Douglas GC. Germination of trinucleated pollen: formulation of a new medium for *Capsella bursa-pastoris*. *Sex Plant Reprod*. 1990; 3(4): 211-215. https://doi.org/10.1007/BF00189949
19. Jayaprakash P, Sarla N. Development of an improved medium for germination of *Cajanus cajan* (L.) Millsp. pollen *in vitro*. *J Exp Bot*. 2001; 52(357):851–855.
20. Vishwakarma PK, Vincent L, Vasugi C, Rajasekharan PE. Effect of cryopreservation on pollen viability, fertility and morphology of different *Psidium* species. *Cryobiology*. 2020; 98: 112-118. https://doi.org/10.1016/j.cryobiol.2020.11.017