https://doi.org/10.17163/abyaups.204.11

Biodiversity conservation and ancestral knowledge: micropropagation of Tzímbalo (*Solanum Caripense*) for sustainable entrepreneurship, Pintag – Ecuador

Misheel Alejandra Carrera
Universidad Particular Internacional SEK, (Ecuador)
Orcid: https://orcid.org/0009-0007-3896-375X

Silvia Noroña Gallo
Universidad Particular Internacional SEK, (Ecuador)
Orcid: https://orcid.org/0000-0001-8404-5596

Katty Coral Carrillo
Universidad Particular Internacional SEK, (Ecuador)
Orcid: https: orcid.org/0000-0002-1680-5650

Introduction

Context of Climate Change and Biodiversity Loss

Climate change represents one of the main threats to global biodiversity, with severe impacts on Andean ecosystems due to their high biological diversity and sensitivity to environmental variations. Factors such as rising temperatures, changes in precipitation patterns, and the expansion of agricultural frontiers have contributed to the degradation of these ecosystems, leading to the loss of native plant species (Calixta et al. 2025; Oro et al. 2024; Urbina & Martinez, 2015).

Importance of Tzimbalo in the Pintag Community

The community of Pintag Amaru is in the city of Pintag in the province of Pichincha, Ecuador, at an altitude of 2850 m.a.s.l at coordinates 0°20’48.6”S 78°24’42.2”W, with a relative humidity of 99%. Located south of Quito, near the Valle de los Chillos, it belongs to a páramo area characterized by the presence of the Antisana volcano and an abundance of streams, making it an important water source. Pintag presents three distinct altitudinal zones: high (3400–3900

masl), middle (3000–3400 masl), and low (2700–3000 masl) (Ministerio del Ambiente, 2023). Agriculture is one of the main economic activities, featuring potatoes and fava beans crops in the high zone, corn and beans in the middle, and greenhouse products like tomatoes, celery, and lettuce in the low zone. Biodiversity includes endemic plant species threatened by agricultural expansion and livestock farming. In the lower zone, family-managed gardens produce vegetables and medicinal plants, many of which are underutilized due to lack of resources and technical assistance (Guayasamín, 2023; Lizano, 2015). The Tzimbalo (*Solanum caripense*) is a plant species distributed throughout the country and adapts to humid areas at altitudes between 2200 and 3000 m.a.s.l (Ministerio de Agricultura y Ganadería, 2022). Its fruit, a juicy berry high in sucrose, vitamin C, and minerals, not only provides significant nutritional value for humans but is also a valuable resource for agricultural biodiversity (Rodríguez-Burruezo, 2003). However, its survival is threatened by urban expansion and habitat degradation; studies have reported its presence in only two (2) out of ten (10) evaluated locations in Quito, suggesting a concerning reduction in its populations (Herráiz, 2015). Tzimbalo has been used in hybridization programs with pepino dulce (*S. muricatum*), demonstrating its potential in crop genetic improvement and its importance within agrodiversity (Morales & Vaca, 2016).

Despite this, there is limited information about its cultivation and propagation, reinforcing the need for research that enables its conservation and sustainable use, ensuring its continued presence in ecosystems. In 2013, through the Community Mediation area, the Fundación de Museos de la Ciudad aimed to establish connections with neighborhoods and communities to create, together with the museums, living spaces for collaborative processes through the codesign of cultural projects and action research. Within this framework, and as part of the "Museo Interactivo de Ciencia" program, work was carried out with the communities of Pintag Amaru and La Toglla, where it was identified that the fruit of the Tzimbalo species (*Solanum caripense*) is considered by the community as a spiritually powerful element, used for approximately 300 years in ancestral medicine. This fruit is employed in healing rituals to treat what is known as "espanto" (evil eye -"mal de ojo"), and negative energies in children, and is considered as a fruit of power used by the "Mamas" and "Taitas" in spiritual cleansings (Museos de la Ciudad, 2023). Field research determined that this species is currently found in the home of a resident in the Valencia neighborhood, located 2.5 km from the center of Pintag, and is the only living plant registered. In response to this situation, this research aims to develop a propagation protocol for Tzimbalo (*Solanum caripense*), using plant-based technologies to harness its properties through *in vitro* cultivation to identify the microbiological contaminants that have led to its disappearance in the areas of Pintag and Ilaló. In this

way, the study seeks to contribute to the development of sustainable entrepreneurship linked to the preservation of ancestral knowledge and beliefs.

Theoretical Botanical Description of Tzimbalo

Tzimbalo (*Solanum caripense*) is a plant native to the Ecuadorian Andes that stands out for its ecological versatility and cultural relevance. Its small, sweet-and-sour fruits are rich in antioxidants and vitamins, making them an ideal raw material for the production of preserves, jams, juices, and gourmet sauces (Herráiz, 2015). Additionally, the leaves and roots of the plant possess medicinal properties recognized in traditional medicine, such as their use to relieve digestive ailments and strengthen the immune system (Verdezoto, 2017). These characteristics make it valuable not only from a nutritional perspective but also as a potential resource for functional and natural products (FAO, 1996). Moreover, Tzimbalo has regenerative properties for degraded soils and serves as food for pollinators, reinforcing its importance in local biodiversity. These attributes position Tzimbalo as a strategic plant for social development and entrepreneurship in rural communities such as Pintag, integrating ecological conservation with sustainable economic opportunities through plant-based technologies (Hartmann & Kester, 1998; Vásquez, 2021).

According to interviews conducted with local farmers during a general assembly, as part of this research, the decline of Tzimbalo in the community of Pintag reflects the effects of climate change and agricultural practices that have altered its natural habitat. Factors such as the loss of fertile soils, competition with commercial crops, and the absence of conservation strategies have led to a reduction in its population. This situation affects local biodiversity and limits opportunities for the community to continue benefiting from this plant due to livestock exploitation in the territory, affecting the soil putting their ancestral and economic heritage at risk (D. Acosta, personal communication, October 17, 2024), (Andes Ecuador, n.d.). Knowledge management and innovation enable the integration of scientific and traditional knowledge with creative strategies, fostering the development of entrepreneurs in management and conservation who can enhance the market value and sustainability of such resources. This process is strengthened through innovation networks, such as the collaboration between universities, communities, and businesses for the development of new products. This will allow for the creation of a replicable model in other communities, generating both economic and environmental impact (Chirinos, Pérez, Martínez de Meriño & Meriño, 2017; Noroña, Cajas-Cajas, Revelo-Oña & Cabrera-Cedeño, 2024).

Materials And Methods

In Vitro Cultivation

According to Herráiz (2015), *in vitro* plant cultivation involves isolating plant organs, tissues, or cells and adapting the appropriate conditions to induce physiological responses and obtain microbe-free plants in an aseptic (sterile) nutrient medium through explants. Tissue culture techniques have various applications and have become a valuable tool in multiple fields. *In vitro* plant cultivation is ideal for investigating processes such as cell differentiation and morphogenesis. *In vitro* micropropagation techniques have proven highly useful for the protection of threatened flora, successfully enabling the propagation of several endangered and/or endemic species for their rescue or *in vitro* germination processes, such as tropical orchid species (Luna et al. 2016; Sáez & Roselló, 2013). Currently, *in vitro* cultivation techniques have been consolidated as key tools for the development, conservation, and improvement of fruit species from subtropical climates (Cárdenas, Romero, Echeverri, & Pacheco, 2023), including blueberry (*Vaccinium* spp.), banana (*Musa* spp.), and mortiño (*Vaccinium floribundum*), among others (Brenes Angulo, Castillo Matamoros, & Gómez-Alpízar, 2015). These techniques make it possible to address challenges such as mass propagation, germplasm rescue, and the preservation of genetic diversity. The most used methods include bud, meristem, and zygotic and somatic embryo cultures due to their effectiveness in ensuring the regeneration and genetic stability of propagated plants.

Collection and Preparation of Solanum caripense *Samples*

The collection site was in the Valencia neighborhood, 2.5 km from the center of Pintag, at the home of a resident designated by the Pintag Amaru community to preserve the Tzimbalo species, being the only known living plant in the area. Two field trips were conducted during the last quarter of 2024, during which plant tissue samples were collected from the mother plant of *Solanum caripense.* Longitudinal cuts were made using pruning shears, harvesting approximately 12 branches, each approximately 30 cm in length. The samples comprised leaves ranging from 2 to 5 cm in size, from which explants containing axillary and apical buds (1–2 cm in length) were selected for propagation. These were transported in Ziploc bags at room temperature to the Environmental Laboratory of UISEK University, where they were stored at 3.6 °C to maintain humidity and prevent dehydration (Jiménez, Barrera, Huachi, Vera, & Caicedo, 2020). The extracted material underwent two distinct processes: the first in the disinfection area and the second in the laminar flow hood (Murashige, 1962). The materials were placed in the laminar flow hood under ultraviolet (UV) light for 20 minutes to ensure surface sterilization.

Simultaneously, the disinfection of the explants was carried out by varying the concentrations of the reagents and exposure times, as described in the protocols by Suquillo (2016). Five disinfection treatments were evaluated: T1, T2, T3, T4, and T5. The explants were cut into 1.5 cm sections containing buds and placed in 50 mL of distilled water. In disinfection treatment 1 (T1), ten drops of Tween 20 were added to the solution. Two consecutive rinses with distilled water were performed before transferring the explants to the laminar flow hood, where they were immersed in 50 mL of a 2% NaCl solution. Finally, two additional rinses were carried out using sterile distilled water. In treatment T2, the explants were disinfected with 0.5% NaClO, followed by rinsing with distilled water and 70% ethanol in the laminar flow hood. Treatment T3, 1% NaClO was used, followed by 70% ethanol and rinsing with sterile distilled water in the laminar flow hood. In treatment T4, the explants were treated with 100% and 1% povidone, then with 2% NaClO, and finally rinsed with sterile distilled water. In treatment T5, Skul 27 fungicide was applied, followed by 5% NaClO and a final rinse with sterile distilled water in the laminar flow Hood.

Table 1
Disinfection process by treatment

Treatment	Reagent	Time (min)	Laminar flow hood
T1	Tween 20	10	NaClO 2% + 2 Ad estéril
T2	NaClO 0.5%+ 2 Ad	15-30s	Alcohol 70% + 2 Ad estéril
T3	NaClO 1% + 2 Ad	10-30s	Alcohol 70% + Ad
T4	Povidin 100% + Ad + Povidin 1%	10	NaClO 2%+ 2 Ad estéril
T5	Skul 27 + H2O + NaClO 5% +Ad	10	Ad estéril

Planting in laminar flow hood

According to the protocol described by Suquillo (2016), explants were planted in a basal culture medium. The medium was prepared by adding 30 g of sucrose, 8 g of agar, and 4.4 g of Murashige & Skoog salts, with the pH adjusted to 5.8. The prepared medium was then poured into 250 mL glass jars, each containing 30 mL of the medium, and sterilized in an autoclave at 121°C and 1 atm of pressure for 45 minutes. For planting, four explants were placed in each jar, sealed with plastic film, and transferred to a controlled environment chamber. A total of 90 glass jars were used, with 160 explants distributed among them. Two trials were conducted: the first with 70 explants and the second with 90. The explants were maintained under a photoperiod of 14 hours of light and 8 hours of darkness, using white LED lighting, as indicated by Morales & Vaca (2016).

Results

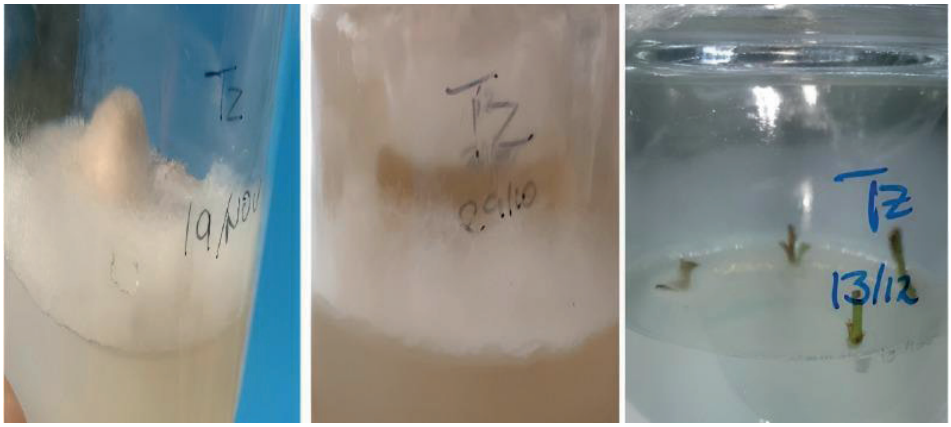
A total of 160 explants were obtained from the 12 branches. In all trials, the presence of filamentous fungi was observed and identified as belonging to the genus *Fusarium* and *Aspergillus* through Gram staining techniques, as illustrated in Figure 01. Contamination persisted in the plant tissue even after disinfection treatments, appearing at 48 and 120 hours after inoculation. The presence of fungi in explants from woody plants has been previously reported, particularly in mature specimens cultivated in open fields (Villalobos, Santos, Isea, 2000).

Figure 1
Solanum caripense *explants showing fungal contamination within 48 hours of inoculation. Mycelial growth is observed around the base of the explant.*



During the second field collection, visual signs of fungal infection were observed on the plant. As illustrated in Fig.2, these findings suggest that contamination may be related to the time of collection and the surface disinfection technique applied (Amin & Jaiswal, 1987), and that the fungi may be endogenous, forming symbiotic associations such as mycorrhizae.

Figure. 2
Contamination observed at 120 hours post-inoculation, identified as Fusarium spp. by Gram staining. Colonization occurs in both the middle and tissue of the explant.



Discussions

The micropropagation of *Solanum caripense* revealed persistent fungal contamination, particularly by genera such as *Fusarium* and *Aspergillus,* even after applying various disinfection protocols. This phenomenon aligns with findings by Villalobos et al. (2000), who identified similar challenges in nodal explants of woody species. One possible explanation is the endophytic nature of these fungi, which inhabit plant tissues without causing visible symptoms. White et al. (2019) suggest that endophytes can be an essential part of the plant microbiome, influencing stress responses, pathogenic resistance, and overall plant development. Furthermore, Schulz and Boyle (2005) describe the "endophytic continuum," highlighting the dynamic relationship between host and symbiont, which may shift from mutualistic to pathogenic, depending on environmental or physiological conditions.

Acknowledging this possibility opens the door to future studies aimed at characterizing these microorganisms using molecular tools such as ribosomal ITS DNA sequencing to determine whether their role in the plant life cycle is mutualistic, commensal, or pathogenic. Moreover, understanding the ecology of these endophytes could support the design of more respectful and effective disinfection strategies, avoiding the inadvertent removal of beneficial microbes (Choi & Kim, 2022; Compant et al. 2016). Alternative approaches may include the use of selective antimicrobials or the development of controlled microbial consortia to enhance culture viability without sterilizing the plant holobiont.

The results obtained (Figures 1 and 2) reveal persistent fungal colonization, with mycelial growth appearing between 48 and 120 hours after inocu-

lation, despite the application of various disinfection protocols. This pattern suggests that the contaminants may have an endogenous origin, possibly forming part of the plant's internal microbiome. These findings align with the concept of the plant as a holobiont, as proposed by Van der Heijden and Hartmann (2016), which considers the plant and its endophytes as a single ecological unit. From this perspective, conventional disinfection methods may inadvertently eliminate potentially beneficial symbionts, disrupting explant physiology and reducing *in vitro* culture success. Studies by Hardoim et al. (2015) and Cassells & Curry (2001) emphasize the need for more targeted strategies, such as the use of selective antimicrobial agents in culture media or the design of controlled microbiomes (Rasmussen et al. 2021).

In this context, somatic embryogenesis emerges as a promising alternative propagation pathway. According to Sáez and Roselló (2013), somatic embryogenesis can be employed through embryo culture, enabling regeneration from cells that are less exposed to endophytic colonization. Recent research on native Latin American species has demonstrated that this technique significantly reduces initial contamination rates (Martínez et al. 2021). Moreover, as Loyola-Vargas and Ochoa-Alejo (2016) emphasize, somatic embryogenesis provides a highly effective platform for plant regeneration, genetic stability, and pathogen-free material production, particularly in recalcitrant or contaminated species. In addition, temporary immersion systems (TIS) have also been suggested as viable alternatives to reduce contamination and improve nutrient absorption (Etienne & Berthouly, 2002), offering further opportunities for optimization.

Moreover, the integration of community knowledge has been essential not only in identifying viable explant sources but also in proposing alternative ecological disinfection techniques. The intersection between ancestral practices and scientific experimentation offers a culturally respectful and biologically effective framework for species conservation. This approach aligns with the agroecological co-innovation model advocated by Altieri et al. (2015), which highlights the key role of rural communities in the development and adaptation of sustainable technologies.

Conclusions

This study indicates that the loss of the *Solanum caripense* species is due to fungal contamination, specifically identified as belonging to the genus *Fusarium*. This finding suggests that the plant material carries an intrinsic microbiological load, likely due to its growth under open-field conditions. Therefore, it is proposed to carry out micro-propagation using seeds and evaluate whether its development reaches an exponential growth phase.

Variability was observed in the effectiveness of the disinfection treatments. The different treatments displayed heterogeneous levels of efficacy, with higher fungal proliferation recorded in those using lower concentrations of sodium hypochlorite (NaClO). The images obtained reveal fungal colonization on the culture medium, negatively impacting the micropropagation process of *Solanum caripense*. These findings indicate that, with adjustments to the disinfection protocols and the implementation of pre-treatments using organic antifungals or osmotic agents, the survival and regeneration of explants could be improved in future cultivation stages.

The fungal contamination detected in the samples suggests the need for *in situ* pre-treatment with fungicides, preferably of organic origin, as recommended by the local community, to avoid harming the soil and other plant species. It is anticipated that a well-formulated solution of this kind could benefit not only Tzimbalo but also other open-field plants facing similar fungal issues, such as manzana de monte and zanahoria blanca, which were also examined in this study.

Finally, it is recommended to develop natural-origin bio-inputs to support regenerative agriculture through the combination of different plant and fungal species from local biodiversity. The goal is to create a biofungicide capable of eliminating or inactivating the fungus without dehydrating the plant, thus helping the species combat present pests. The microbiological analysis of the fungi identified in this research can serve as a basis for future initiatives aimed at developing innovative solutions.

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