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## WATER AND MOORLAND DYNAMICS: IMPACTS AND CONSERVATION STRATEGIES

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### MISCELLANEOUS

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# *Editorial*

Dear reader,

The Andean páramos occupy less than 2 % of the South American continental surface, yet they regulate a significant portion of the water supply that sustains more than 60 million people downstream. Over the past decade, these ecosystems have transformed from "lands of mist" into real natural laboratories where high-altitude ecology, community water management, and environmental governance converge. Aware of this historical moment, *La Granja, Journal of Life Sciences*, presents a Special Issue featuring research that illuminates diverse aspects of the páramo–water dynamic and outlines pathways for its conservation and sustainable use.

The first contribution, from Colombia, titled "*Characterization of Andean Wild Fruit Species as a Strategy for Environmental Conservation in the Western Colombian Páramo*" by Manuel Galvis Rueda and Manuel Torres, documents 48 fruit species in 14 botanical families. It demonstrates how ethnobotanical knowledge can be integrated with páramo-guardian programs and community ecotourism to mitigate degradation caused by mining and intensive livestock farming. The findings confirm that conserving plant diversity not only safeguards key hydrological functions—such as water storage and release-but also diversifies local livelihoods, a critical element in the face of climate change.

From the perspective of engineering and water policy, the article "*Analysis of Sustainability Challenges in Andean Irrigation Systems*" by Gina Berrones and Rolando Célleri analyzes 235 irrigation systems in Ecuador and identifies structural bottlenecks: disproportionate water allocations, weak regulatory enforcement, and increasingly irrelevant sources of agricultural income. The study shows that water sustainability does not depend solely on physical supply, but rather on robust institutional arrangements. Governance thus emerges as an "invisible infrastructure" that ensures efficiency and equity in water use.

Complementing this approach, the study "Community Management and Sustainability in Andean Irrigation Systems through Efficient Water Use Indicators" by Charles Cachipuendo, Mercy Ilbay, and Narcisa Requelme, employs the MESMIS methodology and the Delphi technique to develop 31 indicators aligned with the SDGs and the Principles for Responsible Investment in Agriculture. The result is a locally grounded tool that empowers water boards to audit their own performance and negotiate public policy with evidence in hand. This is a vital contribution, as it translates global frameworks into locally comprehensible metrics for community leaders, technicians, and policymakers.

Together, the articles in this issue demonstrate that the hydrological resilience of the páramos is rooted in biocultural diversity: protecting wild fruit species is as strategic as monitoring streamflows. Moreover, the sustainability challenges of irrigation systems are not purely hydraulic; they require clear rules, appropriate incentives, and informed participation. Measuring matters: without shared indicators, dialogue among communities, academia, and the state remains stalled in perceptions.

From our Miscellanea Section and venturing into the field of Biotechnology, Elias Mjaika Ndifon from the Faculty of Agriculture at Alex Ekwueme Federal University Ndufu-Alike, Nigeria, evaluates the effectiveness of plant extracts and antibiotics in controlling bean blight caused by *Xanthomonas axonopodis*. Developed under laboratory and greenhouse conditions, the study shows that antibiotics such as tetracycline, lincomycin, and erythromycin achieve inhibition levels above 50 %, while extracts from *Eucalyptus globulus* and *Aframomum melegueta* also exhibit significant bactericidal effects, proposing new phytosanitary management approaches.

From Ecuador, Jaime Naranjo-Morán (Universidad Politécnica Salesiana, UPS), Karen Olivo-Fernández, Milton Barcos-Arias, and Rodrigo Oviedo-Anchundia (all from Escuela Superior Politécnica del Litoral, ESPOL), with Barcos-Arias also affiliated with Universidad Espíritu Santo (UEES), present a study on selecting trap plants and suitable substrates for the production of mycorrhizal inoculum. The work identifies *Plectranthus tomentosa*, cultivated in a mixture of sand, rice husk, and peat, as the species with the highest mycorrhization and sporulation, contributing to biotechnological advances in the propagation of arbuscular mycorrhizae.

Likewise, from the University of Mosul in Iraq, Dr. Ali Adel Dawood investigates the therapeutic potential of the TRK inhibitor Vitrakvi (Larotrectinib) in treating solid tumors carrying NTRK gene fusions. Using molecular docking techniques, the author analyzes the drug's interaction with TRK receptors, particularly in the presence of TRKC mutations, revealing structural changes that may affect its efficacy. This work provides a valuable contribution to the development of targeted, personalized therapies in molecular oncology.

From the Earth Sciences domain, the research team from the Universidad Técnica del Norte (UTN), the researches Paúl Arias-Muñoz, Evelin Chamorro-Benavides, Sandy Patiño-Yar, Gabriel Jácome-Aguirre, and Oscar Rosales, examine the combined effects of land use change and climate change on the potential distribution of sugarcane in the Chota Valley, Ecuador. Based on land cover projections and climate scenarios RCP 4.5 and RCP 8.5, the study reveals a 14.65 % reduction in cultivable area due to anthropogenic factors, but also a projected increase in optimal zones under future climate conditions, emphasizing the complexity of agricultural planning in a changing environment.

In the field of Agricultural Sciences, Lucero Perera Hau, Juan Carlos Alamilla, Tomás Gonzales Estrada, and José Humberto Caamal of the Colegio de Postgraduados Campus Campeche (BIOSAT), along with Alberto Santillán Fernández (IxM-CONAHCyT) and Norman Aguilar Gallegos (Universidad Panamericana, Mexico), conduct a meta-analysis of scientific research on *Carludovica palmata* Ruíz Pavón, a species traditionally used in hat-making. The study shows that despite growing interest in craft transformation and traditional production, there remains a marked gap in propagation techniques-especially *in vitro*-highlighting a crucial issue in meeting increasing market demand.

Within Environmental Sciences, researchers from the Faculty of Engineering and Applied Sciences at Universidad Técnica de Manabí, including Josseline Solís Bermúdez, Gabriela Zambrano Varela, Dr. Ramón Eudoro Cevallos Cedeño, and María Antonieta Riera, analyze the potential of various agricultural residues for the establishment of a small-scale biorefinery. Through a multicriteria approach using the AHP method, they identify sugarcane bagasse and coffee husk as the most promising biomasses for producing biofuels and chemical products, reaffirming the relevance of bioeconomy in the sustainable utilization of agro-industrial waste.

Finally, a multidisciplinary team from Escuela Superior Politécnica del Litoral (ESPOL) and Universidad de Guayaquil, led by Tatiana Zamora, along with Joel Vielma-Puente, Luis Galarza Romero, Meribary Monsalve, Joan Vera, Viviana Corrales, Fernanda Chacha, Darling Balón, Leticia Villacis, and Rodrigo Espinoza, presents a study on the production of bioethanol from cocoa shells (*Theobroma cacao*). The process integrates alkaline pretreatment, enzymatic hydrolysis using *Trichoderma reesei* and *T. ghanense*, and alcoholic fermentation with Saccharomyces cerevisiae, resulting in a promising output of bioethanol, relevant to sustainable energy transitions.

We therefore invite our readers to explore this special issue through an interdisciplinary lens. You will find robust empirical data, replicable methodologies, and above all, examples of collective action that transcend national borders. We trust that this issue will inspire new collaborations so that, in the páramos, water may continue to flow not only through the streams but also through science and the hope of those who depend on it.

Sincerely,

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*Special Issue / Edición Especial*
DYNAMICS OF WATER AND THE PÁRAMOS

# CHARACTERIZATION OF ANDEAN WILD FRUIT TREES AS AN ENVIRONMENTAL CONSERVATION STRATEGY IN THE EASTERN MOUNTAIN RANGE OF PÁRAMO, COLOMBIA

## CARACTERIZACIÓN DE FRUTALES SILVESTRES ANDINOS COMO ESTRATEGIA DE CONSERVACIÓN AMBIENTAL EN EL PÁRAMO DE LA CORDILLERA ORIENTAL, COLOMBIA

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**Abstract**

The páramos are ecosystems between 3,2000 and 4,900 meters above sea level. Currently, in Boyacá, the páramos are undergoing mining, deforestation, cattle ranching, and conservation of various plant species, as well as the declaration of nature reserves that strengthen nature ecotourism. The objective of the research was to characterize trees, shrubs, and vines of native fruit tree species, as well as to record the status of the use of fruit-type plant species and the knowledge held by the community in the department of Boyacá. Transect methodologies were applied, with the creation of square plots according to the type of forest and shrub cover. Additionally, an inventory of fruit tree species was conducted, along with photographic records with identification by expert taxonomists and specialized botanical catalogs. As a result, 48 species were recorded in 14 botanical families among the identified plants. The most diverse family in number of genera and species is Ericaceae with 12 species, Rosaceae with 8 species, Solanaceae and Passifloraceae with 3 species, Myrtaceae with 2 species and 6 families with one species. Likewise, the categorization and community information processes were systematized in categories of ethnobotanical use, for example: medicinal, tinctures, juices and conservation. Other species with nutritional functions were identified, for which a descriptive photographic botanical catalog was prepared. Conclusion: It is important to educate the community, as well as organize paramo rangers, to allow the conservation, diversification and use of products and by-products of the páramo.

***Keywords***: Biodiversity, native fruits, productive systems, botany.

**Resumen**

Los páramos son ecosistemas comprendidos entre 3200 m.s.n.m, y 4900 m.s.n.m. Actualmente, en Boyacá se presentan en los páramos procesos de explotación minera, deforestación, ganadería y conservación de varias especies de plantas, así como declaratorias de reservas naturales que permiten fortalecer el ecoturismo de naturaleza. El objetivo de la investigación fue realizar la caracterización de árboles, arbustos y lianas de especies de frutales nativos, además registrar el estado del uso de especies vegetales de tipo frutales y del conocimiento que tiene la comunidad en el departamento de Boyacá, se aplicaron metodologías de transeptos, con realización de parcelas cuadradas, según el tipo de cobertura de bosques y arbustales. Adicional, se realizó el inventario de las especies de frutales, el registro fotográfico con la identificación por expertos taxónomos, catálogos especializados en botánica. Como resultado; se registraron 48 especies en 14 familias botánicas, entre las plantas identificada. La familia más diversa en número de géneros y especies es ericaceae con 12 especies, rosaceae 8 especies, solanaceae y passifloraceae con 3 especies, myrtaceae con 2 especies y 6 familias con una especie. Así mismo se sistematizaron los procesos de categorización e información comunitaria en categorías de uso etnobotánico, ejemplo: medicinal, tinturas, jugos y conservación identificaron otras especies con funciones alimentarias por lo que se elaboró un catálogo botánico fotográfico descriptivo. Conclusión; es importante educar a la comunidad, así como organizar guarda páramos, para permitir la conservación, la diversificación y el aprovechamiento de productos y subproductos del páramo.

***Palabras clave***: Biodiversidad, frutas nativas, sistemas productivos, botánica.

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# 1 Introduction

Plant diversity in the paramo is a key element to be characterized, despite the fact that, as noted by Hofstede et al. (2003), the number of genera and species identified as endemic in paramo ecosystems is relatively low. However, the known values for the number of endemic species are significantly high. One possible explanation for this phenomenon is the relatively recent geological emergence of this ecosystem, which may have led to specialization at the species level rather than at the genus level. For example, the ericaceae family in neotropical forests is among the most representative, mainly inhabiting montane forests. It is currently represented by approximately 46 genera and 900 species, with about 94 % of them being endemic (Kron et al., 2002).

This research aims to characterize and document native fruit-bearing plants and proposes strategies for their conservation within Andean systems. Fieldwork methodologies included the establishment of twelve square plots for plant inventories, taking into account shrub and forest cover across different altitudinal transects in the Guantiva-La Rusia paramo complex, located in the western corridor of Boyacá.

Among other aspects, the study addresses the need to identify wild native fruit species that play a fundamental role in ecosystem dynamics and are associated with specific territorial areas. This implies that the ecological benefits of these fruit species promote ecosystem balance and serve as a food source for members of food webs, such as consumers (Parada-Quintero et al., 2012). Therefore, this descriptive and experimental research will enhance opportunities for collaboration with local rural communities in the paramos.

Fruits are among the most commonly consumed food items both in the rural areas under study and globally, particularly in the Andean region of Latin America (Sanjinés and Øllgaard, 2006), as exemplified by the ericaceae family, whose distribution aligns with the findings of the present study.

# 2 Materials and Methods

This research was conducted to characterize and document native Andean fruit species with the goal of promoting their conservation. A mixed-methods approach and a systematic descriptive methodology were adopted, establishing twelve inventory plots based on vegetation research methods by Rangel and Velázquez (1997). These plots considered shrub and natural forest coverage along altitudinal transects in the Guantiva-La Rusia paramo complex, covering the municipalities of Cómbita, Sotaquirá, Paipa, and Duitama, Boyacá.

The study titled *"Characterization of Wild Fruit Species as a Conservation Strategy for the Páramo in Boyacá"* involved inventory and documentation, including photographic records of the species and their fruits. To identify their uses, direct dialogue with communities near the paramo was essential (Galvis-Rueda and Torres-Torres, 2017).

## 2.1 Research Approach:

The investigation was based on a mixed-methods design, representing a set of systematic, empirical, and critical processes involving the collection and analysis of both quantitative and qualitative data. This integrated analysis enhances the understanding of the studied phenomenon (Hernández-Sampieri and Mendoza, 2018). According to Creswell (2013) and Lieber and Weisner (2010), as cited in Hernández-Sampieri and Mendoza (2018), mixed methods incorporate numerical, textual, and visual data to explore complex problems.

## 2.2 Research Paradigm:

The research was grounded in a socio-critical paradigm, characterized by self-reflection and driven by community needs as the basis for knowledge construction (Alvarado and García, 2008).

## 2.3 Study Population:

A sample of 80 adult participants was convened, 20 from each municipality. In Cómbita, participants were from the Laguna community and El Valle Regional Park; in Sotaquirá, from the central area and Avendaños village; in Paipa, from Los Medios and Palermo villages, including Ranchería Municipal

Park; and in Duitama, from communities bordering the Andalucía Forest Reserve. The Tibairá Corporation participated with five members, alongside 15 farmers from La Quinta and Santana villages, all of whom carry out agricultural activities in the paramo under study.

## 2.4 Research Phases:

**Phase 1 –Action Plan Formulation:**

Following Latorre (2003), an action plan was developed with flexible and context-adaptable components for information gathering and critical reflection.

**Phase 2 –Botanical Identification of Fruit Trees:**

Collected specimens were dried and identified at the Instituto de Ciencias Naturales (ICN) of the National University of Colombia or at UPTC in Tunja. Taxonomic identification followed Cronquist (1981) and used sources such as the Alexander von Humboldt Virtual Herbaria. Each specimen received a collection label with essential field data. Identifications were verified with experts using the most current taxonomic revisions and the Colombian National Herbarium (COL), accessed virtually.

**Inventory and Cataloguing:**

A photographic catalog of fruit species in the natural reserves and study area was compiled to facilitate recognition. This included ethnobotanical information on local uses, morphological and botanical descriptions, among other traits.

**Phase 3 –Ethnobotanical Use Analysis:**

A detailed activity plan was implemented, incorporating ethnobotanical categorization based on the Kew-Colplanta database (Sánchez et al., 2021). Twenty interviews were conducted in each municipality (80 in total), targeting adult residents with influence in the forest reserves. Interviewees identified wild fruit species and their uses—such as food or pigment extraction—contributing to a more comprehensive catalog. The work was supported by specialized texts like *Descriptive Botany of Tropical Crops* (León, 1987).

**Phase 4 –Field Observation and Community Engagement:**

Field observations were conducted on farms to gather evidence and assess direct dialogue with local farmers. These observations were planned and systematically recorded to address the core research questions, particularly concerning paramo preservation challenges identified by the community.

**Phase 5 –Participatory Reflection and Conservation Planning:**

Following the Action Research model (Kemmis and McTaggart, 1988), reflection focused on evaluating the outcomes of community-driven conservation efforts. The study led to the proposal of a mid-term conservation strategy: the design of a nursery in the Duitama–Río Surba region. This initiative aims to support the propagation of native species as a response to the impacts of climate change and frequent wildfires in high mountain ecosystems.

## 2.5 Data Analysis:

The qualitative data analysis process followed the framework described by Rodríguez et al. (2005), involving the systematic organization and interpretation of information to identify relationships, meanings, and conclusions.

# 3 Results and Discussion

In the paramo and the study region, a total of 48 species were recorded, grouped into 14 families (Table 1), of these, 99% correspond to species native to Colombia and distributed throughout South America, while 1% are introduced species, either cultivated or naturalized; which coincides with the research of Aguilera-Arango et al. (2020). A total of 45 species were found between 2,900 and 3,600 meters above sea level, each with some traditional use or potential for food or medicinal purposes. Of these, 40 species exhibit potential for ecological restoration and water resource conservation.

From a conservation perspective, several shrub and tree species are proposed as candidates for conservation targets due to their rarity, degree of threat, overexploitation, or endemism. Two species—*Greigia stenolepis* and *Passiflora tripartita*—are documented under the conservation status of Least Concern (LC), though they are categorized as having some level of threat (Morales, 2001).

In comparison, the work by González (2014), *Illustrated Guide to the Plants of the Municipality of Villa de Leyva and Surrounding Areas*, is highly comprehensive, listing 1,293 species of native vascular plants and other exotic species cultivated for edible or ornamental purposes. The current list shares approximately 90 % of its species with González's work, while also contributing new records and ethnobotanical uses relevant to the region of the Eastern Cordillera.

**Table 1.** Ethnobotanical use of families and Species with Edible Fruits from the *Paramo* of the Western Corridor Guantiva-La Rusia, Boyacá.

| Common name | Family | Scientific name | Ethnobotanical uses |
|---|---|---|---|
| Piñuela, piña de páramo | Bromeliaceae | *Greigia stenolepis* L.B.Sm. | Berry fruit; edible seeds, juice preparation, laxative medicinal use. |
| Papayuela | Caricaceae | *Vasconcellea pubescens* (A.DC.) Badillo | Fruit used as food; prepared in various ways including aromatic waters, preserves, and papain production. |
| Guatila | Cucurbitaceae | *Sechium edule* (Jacq.) Sud. | Used as a vegetable, edible fruit and other parts. Phytotherapeutic potential as antibacterial agent. |
| Anise grape | Ericaceae | *Cavendishia bracteata* (Ruiz & Pav. ex J. St.-Hil.) Hoerold | Fruits used for food, sweets, and jams. |
| Camarera grape | Ericaceae | *Macedonia rupestris* (Kunth) A.C. Smith | Fruits used for food, sweets, wine, jams, medicinal and culinary applications. |
| Grape | Ericaceae | *Macleania pubiflora* Benth. | Edible fruit. |
| Small grape | Ericaceae | *Cavendishia pubescens* (Kunth) Hemsl | Edible fruit, potential use in food and cosmetic industries. |
| Paramo grape | Ericaceae | *Disterigma alaternoides* (Kunth) Nied | Edible fruit. |
| Grape | Ericaceae | *Psammisia macrophylla*. (Kunth) Klotzsch. | Edible fruit. |
| Grape | Ericaceae | *Thibaudia floribunda* Kunth | Human food used in juices, sweets, and jams. |
| Grape | Ericaceae | *Gaylussacia buxifolia* Kunth. | Edible fruit in juices, sweets, and jams. |
| Pachim | Ericaceae | *Plutarchia coronaria* (Hook.fil.) A.C.Sm | Fruit used for juice, syrup, and liquor. |
| Agraz, Mortiño | Ericaceae | *Vaccinium floribundum* Kunth. | Human food, used in juices, sweets, and jams. Antioxidant properties. |
| Páramo agraz | Ericaceae | *Vaccinium meridionale* Swartz | Fruit used for juices, jams, sauces, and wines. Antioxidants present. |
| Reventadera | Ericaceae | *Pernettya prostrata* (Cav.) DC. | Medicinal plant, fruit used for tinctures, ethanolic extracts used for anti-inflammatory, antioxidant, and antibacterial properties. |
| Small grape | Ericaceae | *Cavendishia nitida* (Kunth) A.C. Sm | Edible fruit. |
| Grape | Ericaceae | *Cavendishia cordifolia* (Kunth) Hoerold | Edible fruit, used in jams, wines, nectars, juices, candies. |
| Camarera | Ericaceae | *Themistoclesia dependens* (Benth.) A.C. Sm. | Edible fruit. |

| | | | |
|---|---|---|---|
| Camarera grape | Ericaceae | *Psammisia graebneriana* Hoerold | Edible fruit. |
| Barejon, small grape | Grassulariaceae | *Ribes andicola* Jancz. | Edible fruit, used to make jams, also used to treat undiagnosed conditions. |
| Arrayan | Myrtaceae | *Myrcianthes rhopaloides* (Kunth) McVaugh | Fruits and leaves used to prepare colada (traditional beverage). |
| Wild Arrayan | Myrtaceae | *Ugni myricoides* (Kunth) O.Berg | Wild fruit is edible. |
| White Arrayan | Myrtaceae | *Myrcianthes leucoxyla* (Ortega) McVaugh | Edible fruit. |
| Curuba | Passifloraceae | *Passiflora tripartita* (Juss.) Poir. | Edible fruit and pulp used in agro-industrial products like beverages, ferments, ice creams, and juices. |
| Curuba | Passifloraceae | *Passiflora mollissima* (Kunth) L. H. Bailey. | Edible fruit and pulp used in agro-industrial products like beverages, ferments, ice creams, and juices. |
| Canelón | Piperaceaea | *Peperomia subspathulata* Yunck. | Aromatic plant; used in infusions for stomach, head, tooth pain, nerves, bruises, burns. Whole plant used as poultice, purgative, and for deafness. |
| Cherry | Rosaceae | *Prunus serotina subsp. capuli* (Cav.) McVaugh | Edible fruit; used for juices, medicinal, bark, leaves, and fruit have commercial value. Expectorant, sedative, stimulant. |
| Uche, cerezo | Rosaceae | *Prunus buxifolia* Koehne | Fruits are edible (seeds); used as human food. |
| Mortiño | Rosaceae | *Hesperomeles goudotiana* (diciembre.) Killip. | Fruits used for juices, jams, and wines. |
| Mortiño | Rosaceae | *Hesperomeles heterophylla* (Ruiz & Pav.) Gancho | Fruits used for human consumption; juices, jams, wines. |
| Blackberry | Rosaceae | *Rubus urticifolius* Poir. | Fruits used for juices, jams, medicinal, and wine making. |
| Castilian blackberry | Rosaceae | *Rubus bogotensis* Kunth | Fruits used for human consumption; juices, jams. |
| Morón | Rosaceae | *Rubus nubigenus* Kunth | Fruits used for human consumption; juices, jams. |
| Blackberry | Rosaceae | *Rubus robustus* C. Presl | Fruits used for human consumption; juices, jams. |
| Blackberry | Rosaceae | *Rubus compactus* Benth | Fruits used for human consumption; juices, jams. |
| Blackberry, morón | Rosaceae | *Rubus glaucus* Benth | Fruits used for human consumption; juices, jams, medicinal, and wine making. |
| Mortiño | Rosaceae | *Hesperomeles ferruginea* (Kunth) Lindl. | Fruits used for human consumption; juices, jams. |
| Native strawberry | Rosaceae | *Fregaria vesca* L. | Wild strawberry used for food, juices, jams, medicine; alcoholic drinks made with aguardiente. |
| Rojitos, pennyroyal | Rubiaceae | *Nertera granadensis* (Mutis ex L. f.) Druce | Fruit used as food, medicinal, used to treat heart conditions. |
| Native lulo | Solanaceae | *Solanum quitoense* Lam. | Fruit used in traditional beverages like canelazo, colada morada, and chicha. |

| | | | |
|---|---|---|---|
| Goldenberry | Solanaceae | *Physalis peruviana* L. | Food and laxative; supports bone and cartilage health, prevents diseases like osteoporosis; used in traditional drinks. |
| Tomatillo | Solanaceae | *Solanum sisymbrifolium* LAM. | Ripe fruit edible in various preparations; medicinal; ethanolic extract has genotoxic effects. |
| Mashua | Tropeliaceae | *Tropaeolum tuberosum* Ruiz y Pavón | Used in traditional highland cuisine; medicinal use for liver and kidney ailments. |
| Grape vine | Vitaceae | *Cissus alata* Jacq. | Green fruits stewed as vegetable; medicinal use for bruises and hematomas. |
| Paramo chili, cinnamon | Winteraceae | *Drimys granatensis* Mutis ex L.f | Spicy fruit used in food; traditional uses include as a laxative, emmenagogue, and anthelmintic. |

As a result of the inventory, more than 15 tree species were documented (Figure 1), including *Hesperomeles goudotiana* and *Hesperomeles heterophylla* (commonly known as mortiño), *Myrcianthes leucoxyla* (arrayán), and *Prunus serotina* (cherry). Shrub species such as *Macleania rupestris* and *Macleania pubiflora* (uva camarona see Figure 2) species investigated by Acero and Bernal (2003), *Cavendishia pubescens* (uvita), and other fruit-bearing shrubs including *Disterigma alaternoides, Psammisia macrophylla, Thibaudia floribunda, Gaylussacia buxifolia, Plutarchia coronaria, Vaccinium floribundum* and *Vaccinium meridionale* (agraz or mortiño), *Pernettya prostrata* (reventadera), as well as various Rubus species (*R. urticifolium, R. bogotensis, R. nubigenus, R. robustus*, all known as wild blackberries), were recorded.

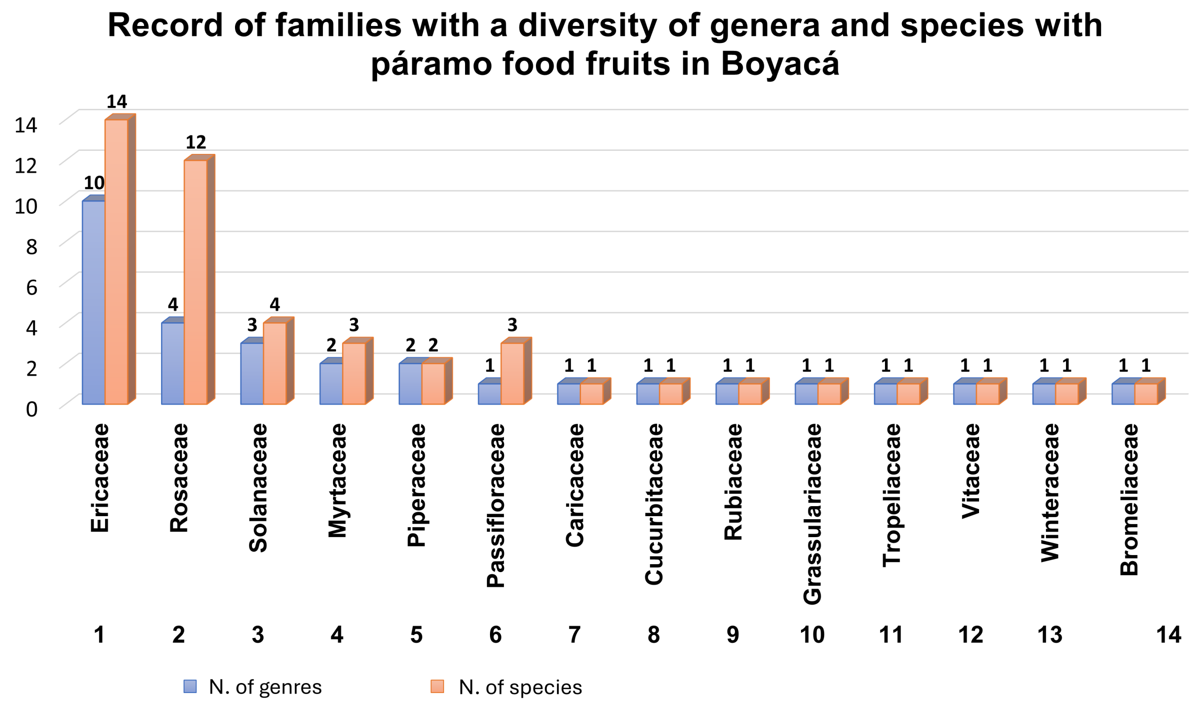


**Figure 1.** Record of Plant Families with Diversity of Genera and Edible Fruit-Bearing Species in the Paramo of the Western Corridor, Guantiva-La Rusia Complex– Boyacá.

Additionally, species such as *Vasconcellea pubescens* (papayuela), *Solanum quitoense* (wild lulo), *Physalis peruviana* (cape gooseberry), *Solanum sisymbriifolium* (tomatillo), *Passiflora tripartita* (curuba) recognized as a promising crop (Casierra-Posada et al., 2017), *Cissus microcarpa* (a fruiting liana), *Sechium edule* (chayote, guatila), *Ribes andicola* (barito from the Grossulariaceae family), and *Greigia stenolepis* (piñuela from the Bromeliaceae family) were included.

This process also involved learning techniques related to fruit collection, seed scarification and preparation, germinator construction, and sexual and asexual propagation (e.g., stem cuttings), specifically for species such as *Drimys granadensis* (paramo chili), *Peperomia subspathulata* (canelón), and *Tropaeolum tuberosum* (cubio, mashua).

The presence of 15 species from the Ericaceae family, whose fruits—particularly those of the genera *Macleania, Cavendishia, Disterigma, Plutarchia*, and *Vaccinium*—are consumed, illustrates a remarkable diversity of edible plants with a long-standing tradition of use in the Duitama region. This cultural practice is closely linked to the traditions of paramo communities, as also documented in Peru by researchers such as Huamantupa-Chuquimaco et al. (2021). In Colombia, these species—commonly known as *uva camarona* or *uva de monte*—are currently being studied for their pulp production potential and transformation into value-added products such as jams and preserves.


**Figure 2.** Photograph and Description of *Uva Camarona* (spanish version) and its use.

In the study of edible fruit-bearing plant species from various families (see Table 1) which coincides with research by Coimbra-Molina (2014), conducted in the western corridor of the Guantiva-La Rusia páramo in Boyacá, it was found that the vegetative cover supports high plant diversity. This includes shrubs, trees, and herbaceous species distributed across 14 botanical families. The family Ericaceae showed the highest genetic diversity with 14 species across 10 genera this coincide with Castro et al. (2023), followed by Rosaceae with 12 species and 4 genera, Solanaceae with 4 species and 3 genera, and both Myrtaceae and Passifloraceae with 2 species each and which hold potential benefit (Rodríguez-Castillo and Melgarejo, 2015). Eight other families were represented by a single genus and species each. These findings align with previous research by Rangel (2000) on plant diversity in Colombia, particularly in high Andean zones and dry to subhumid *paramo* ecosystems, where Ericaceae and Rosaceae dominate due to their high number of genera and species in fruit trees (Fischer et al., 2022).

The Ericaceae and Rosaceae family represent the most diversified groups of plants originating from the Neotropics (Kron et al., 2002). Their diversification is closely associated with specific habitats that require distinct abiotic conditions, which change rapidly along altitudinal gradients. These conditions include variations in soil nutrient availability, precipitation regimes, humidity, temperature, photoperiod, and soil water content, all of which are interrelated with their seed dispersers and pollinators (Cáceres et al., 2014).

In terms of the recorded families and species diversity, the distribution is represented as percentages of the total sample. Plants bearing fruits of alimentary value in the paramo (Quevedo-Rubiano et al., 2021) are dominated by the Ericaceae family, comprising 33%, followed by Rosaceae at 24%, Solanaceae at 9%, Myrtaceae and Passifloraceae each at 7%, and Piperaceae at 4%. Eight additional families -Bromeliaceae, Grossulariaceae, Caricaceae, Cucurbitaceae, Rubiaceae, Tropaeolaceae, Winteraceae, and Vitaceae- are each represented by 2% of the species (Figure 1).

Furthermore, it was confirmed that several of these species play an important role in the conservation of micro-watersheds, as observed in Río de Piedras in Cómbita, Río Sotaquirá, and Río Surba in Duitama, Boyacá. As an Educational Strategy for conservation a Wild Fruit Catalog could be designed. The Figure 2 shows an example with the informative sheet of *Uva Camarona* a kind of wild grape.

## 4 Conclusions

Based on the work conducted, it can be stated that the *paramo* under study functions as a biological corridor with high potential for native fruit biodiversity, especially when compared to other high Andean areas in Colombia. Among the recorded plant families, Ericaceae and Rosaceae are the most prominent, both exhibiting a significant diversity of edible fruits (Diago and Castro, 2021) with various ethnobotanical applications.

The rural communities of the region display a rich pluricultural knowledge base. Through dialogue with these communities, it was established that ethnobotanical uses are primarily managed and transmitted by women (70%), who are also the main users of wild fruits from shrubs and trees -such as agraz (*Vaccinium meridionale*) same contribution by Becerra et al. (2022), lulo (*Solanum quitoense*), anise grape (*Cavendishia bracteata*), curuba (*Passiflora mollissima*), cherry known as *capulí in spanish* (*Prunus serotina subsp*), pachin (*Plutarchia coronaria*), and mortiño (*Hesperomeles heterophylla*) -mainly for the preparation of juices and jams. In contrast, men (30%) tend to use these same plants and other trees for the construction of living fences.

Finally, it is crucial to implement community education programs and to organize local *paramo* guardianship groups to ensure the conservation, diversification, and sustainable use of *paramo* products and by-products, thereby maintaining biodiversity for present and future generations.

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## Authors' contribution

M.G.C.: Conceptualization, data processing, research, methodology, original draft, writing - review and editing. M.T.T.: Conceptualization, research, methodology, project management, writing - original draft, writing -review and editing.

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Dynamics of Water and the Páramos

# Understanding sustainability issues in Andean irrigation systems

## análisis de los desafíos de sostenibilidad en sistemas de riego de los Andes

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**Abstract**

Irrigated agriculture consumes approximately 70 % of the world's freshwater, making sustainable water delivery imperative. Strategies for sustainable water use, incorporating technical, agronomic, managerial, and institutional advancements are urgently needed, especially in developing countries such as those in the Andes, where agriculture is crucial for socioeconomic growth. Identifying the main issues related to sustainability of irrigation systems are essential, but limited information exists, as most studies focus on small groups of systems rather than a diverse range. To address this, we analyzed data from surveys conducted in 2022 by the Regional Government of Azuay, Ecuador, with representatives of water user associations. We adapted the methodology outlined in the United Nations World Water Development Report 2023 to evaluate these mountain irrigation systems, considering socioeconomic, water resources, users and governance factors. Our study included 235 irrigation systems with irrigated areas ranging from 0.5 to 2400 hectares, classified into four groups: (a) micro (<10 ha), (b) Small (10-100 ha), (c) Medium (100-500 ha), and (d) large (>500 ha). The most urgent issues identified include water allocation not proportional to the irrigated area, agricultural production no longer being the primary source of income, and non-compliance with management rules and fee payments. Most of the issues are due to weak governance. Our findings highlight the complexity of irrigation systems and the barriers to their development. This comprehensive analysis provides insights for building effective policies and emphasizes the importance of regular assessments, which should include systematic monitoring, data collection, and the development of performance indicators.

***Keywords***: Water allocation, Governance, Decision-makers, Water User Associations, Irrigated agriculture.

**Resumen**

La agricultura de regadío consume aproximadamente 70% del agua dulce mundial, por lo que resulta necesario asegurar un suministro sostenible. Se requieren estrategias para el uso sostenible del agua, que incorporen avances técnicos, agronómicos e institucionales, especialmente en países en desarrollo en los Andes, donde la agricultura es crucial para el crecimiento socioeconómico. La identificación de temas relacionados con la sostenibilidad en sistemas de riego es esencial, pero la información existente es limitada, ya que la mayoría de los estudios se centran en pocos sistemas. Se analizaron los datos de encuestas realizadas en el 2022 por el Gobierno Provincial del Azuay, Ecuador, con representantes de juntas de riego. También, se adaptó la metodología del Informe Mundial sobre el Desarrollo del Agua 2023 para evaluar estos sistemas, considerando los factores socioeconómicos, recursos hídricos, usuarios y gobernanza. Nuestro estudio incluyó 235 sistemas de riego con áreas irrigadas que varían de 0,5 a 2400 hectáreas, clasificados en cuatro grupos: (a) micro (<10 ha), (b) pequeño (10-100 ha), (c) mediano (100-500 ha) y (d) grande (>500 ha). Los problemas más urgentes identificados incluyen la asignación de agua no proporcional al área irrigada, la producción agrícola que ya no es la principal fuente de ingresos, el incumplimiento de reglamentos y el pago de tarifas. La mayoría de estos problemas se deben a una gobernanza débil. Nuestros hallazgos destacan la complejidad de los sistemas de riego y las barreras para su desarrollo. Este análisis integral proporciona ideas para políticas efectivas y enfatiza la importancia de evaluaciones regulares, que deben incluir monitoreo sistemático, recolección de datos y desarrollo de indicadores.

***Palabras clave***: Asignación de agua, Gobernanza, Tomadores de decisiones, Juntas de riego, Agricultura de regadío.

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# 1 Introduction

Water resources are not only essential for sustaining various sectors of society, including food production, energy generation, and the provision of goods and services, but they also serve as crucial economic drivers in many regions (Sauer et al., 2010; United Nations, 2021). Beyond its essential necessity, the significance of water has become a fundamental factor that influences economic activities and overall societal development. In particular, the way in which agriculture uses freshwater resources is important to ensure availability across diverse sectors and to safeguard ecosystems (FAO, 2020). Agriculture is the largest consumer of water globally and uses approximately 70% of the total water withdrawn for irrigation purposes (United Nations, 2021; Wada et al., 2016; Wisser et al., 2008), highlighting the urgent need for effective management strategies, especially in developing countries where agriculture is pivotal for socio-economic growth (Li et al., 2020), such as in the Andes.

Moreover, agriculture is considered to have the lowest value-added on a global scale, particularly in low- and middle-income countries (United Nations, 2021), which underscores the challenges faced in optimizing its economic contributions. Concerns arise regarding the enhancement of agricultural water productivity while ensuring food security (Bjornlund et al., 2023), particularly in supporting farmers who rely heavily on irrigated agriculture for their livelihoods (United Nations, 2023). Irrigation has emerged as an important driver of yield growth and has played an essential role in facilitating substantial increases in production (Alexandratos and Bruinsma, 2012), thereby underlining its significance in agricultural development.

To achieve sustainable irrigation management, comprehensive strategies encompassing technical, agronomic, managerial, and institutional dimensions are imperative (Gutierrez et al., 2014; Sirimewan et al., 2021a). Irrigation systems involve multiple actors that require interactions between stakeholders to achieve full functionality (van Rooyen et al., 2017). Moreover, irrigation demands extensive infrastructure and expertise to facilitate water access, storage, and conveyance to the schemes, while ensuring equitable distribution to farmers' fields, and environmental needs (Parry et al., 2020). Effective management of water resources, distribution, rights, and maintenance operations involves both local communities and institutional frameworks (van Rooyen et al., 2017).

Social cooperation within communities plays a key role in sustainable irrigation management. Historically, community-managed irrigation systems in the Andes have demonstrated the effectiveness of collective action and collaboration (Hoogesteger, 2015). This is the case of Water User Associations (WUAs), which manage irrigation resources through participatory approaches and have emerged as significant facilitators of collective action, mobilizing communities towards common goals (Hoogesteger, 2013, 2015). Therefore, establishing participatory cooperation among stakeholders is strategic for facilitating water governance and decision-making processes (FAO, 2016; United Nations, 2023).

WUAs often face issues such as inadequate implementation strategies and unclear delineation of roles and responsibilities, among others (United Nations, 2023). These obstacles can significantly impede the effective functioning of WUAs and undermine their ability to achieve sustainable irrigation management goals. One of the most pressing problems is the lack of data and information of all aspects concerning the operation, management and impact of irrigation systems, including the farmers' well-being. There are only a few studies about irrigation management and socioeconomic development in the Andes. Communal et al. (2016) and Hoogesteger (2013) examined changes in water distribution management among small-scale farmers and analyzed community cooperation in managing irrigation systems in the northern Ecuadorian Andes, emphasizing the significance of local initiatives and community engagement in sustainable water resource management practices. Meanwhile, Gutierrez et al. (2014) and Leroy et al. (2022) provided broader perspectives on irrigated agricultural development and institutional changes within WUAs in the Andean region. Leroy (2019) focused on the perception of socioeconomic causes of water scarcity within WUAs in Colombia and Venezuelan páramos. Together, these studies highlight the need for integrated approaches that consider both the local contexts and broader institutional frameworks.

Most existing studies in the Andes have focused on a single or on small groups of irrigation systems, making it challenging to understand the organization, functionality and sustainability of irrigation in the region, especially given the large variations in irrigated area and number of users in those systems. There is limited information on the current physical conditions of irrigation schemes, coordination between users and management, operation and maintenance of the systems, the impact of droughts, and the effect of irrigation on users' livelihoods. Addressing these knowledge gaps is essential for developing comprehensive strategies for sustainable irrigation management in the region.

To our knowledge, no study has focused on a wide range of irrigation systems and how WUAs manage them. To address this gap, this study analyzes and identifies the main sustainability problems faced by users within a large sample of mountain irrigation systems, aiming to provide relevant information to decision-makers for informed interventions.

We adapted the methodology outlined in the United Nations World Water Development Report 2023 (United Nations, 2023), which provides a comprehensive categorization of factors influencing the performance of WUAs and the level of cooperation among stakeholders involved in irrigation systems. While the scope of this study is not to assess the performance of WUAs per se, we used this categorization framework to critically examine the management and sustainability of irrigation systems. We considered the following factors: socioeconomic conditions, water resources, governance, and user dynamics. Our analysis is based on a dataset obtained by the Azuay Regional Government through surveys conducted in 2022 with representatives of 235 WUAs across the province in the southern Ecuadorian Andes.

# 2 Materials and Methods

## 2.1 Study Region: Mountain irrigation systems of Azuay

The Azuay Province, located in southern Ecuador, encompasses two zones: the Inter-Andean region, limited by the western and eastern Andean cordilleras, and the western coastal region (Tenesaca et al., 2017). Covering an area of 8,309.6 $km^2$, Azuay exhibits significant bioclimatic diversity, resulting in seven distinct bioclimatic zones. These include ecosystems, such as páramo grasslands, evergreen montane forests, and areas influenced by human activities (Tenesaca et al., 2017). The regional climate is marked by considerable variability. Research in the Paute Basin demonstrates significant spatiotemporal differences, particularly in precipitation, where mean annual totals range from approximately 660 mm in inter-Andean valleys to over 3400 mm on eastern cordillera slopes receiving substantial Amazonian moisture (Celleri et al., 2007). Temperature also shows significant variation with altitude and exposure, creating diverse thermal conditions from cold, humid páramos to milder lower valleys (Campozano et al., 2016; Celleri et al., 2007).

The provincial territory is predominantly characterized by steep slopes, which limit agricultural mechanization, particularly owing to irrigation challenges. There are risks of water and wind erosion, as well as soil mass movement. Less than 15% of the provincial territory consists of plains and rolling hills, which are generally stone-free and suitable for various types of agricultural mechanization, with some restrictions (GPA, 2018).

The rural areas of Azuay are known for their agricultural and livestock economies (GPA, 2018). In Azuay, agricultural practices are mainly focused on self-consumption and are frequently combined with other non-agricultural activities (GPA, 2019). The III National Agricultural Census highlights the cultivation of various crops in Azuay, including intercropping of maize and bean, potatoes, broad beans, peas, carrots, and diverse fruit trees. These agricultural practices largely use traditional methods with minimal technological implementation (GPA, 2019). In terms of cultivated area, Azuay covers approximately 205,281 hectares dedicated to agriculture and livestock (ESPAC, 2023). The irrigation systems in the province are public, communal and private, which are often managed by WUAs.

## 2.2 Data sources

The data used in this study were obtained from the inventory of irrigation systems conducted by the provincial government of Azuay in 2022. Prior to this inventory, the available information pertaining to irrigation systems within the province was scattered across various sources and governmental institutions, thus lacking a consolidated, complete and coherent dataset. In line with the strategic objectives of the provincial irrigation plan, the task of data collection was undertaken to formulate policies to ensure effective water management specifically for irrigation purposes. In addition, the design of the survey involved collaboration among an interdisciplinary team from the local government, a non-governmental organization, consultants and the University of Cuenca.

While approximately 400 irrigation systems have been reported to be operational within the province, the information at hand pertains solely to 267 irrigation systems. Furthermore, of these 267 systems, 168 irrigation systems have been georeferenced, as depicted in Figure 1. Certain data gaps emerged across various questionnaire topics stemming from respondents' limited access to information and logistical challenges. Considering these factors, we specifically used a dataset of 235 irrigation systems, ensuring a reliable foundation for the analysis.

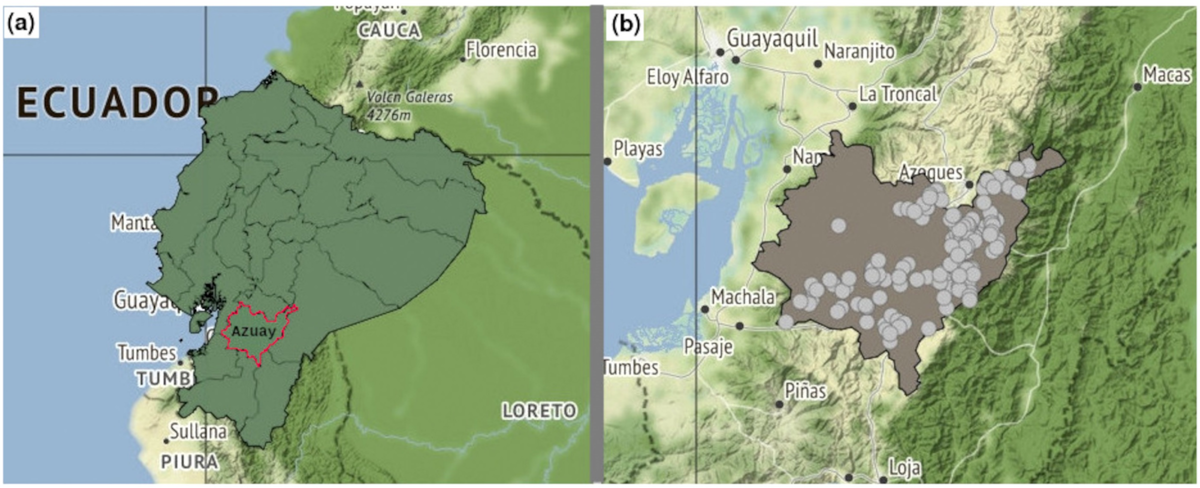


**Figure 1.** (a) Location of Azuay Province in Ecuador. (b) Irrigation systems in Azuay Province. The colored dots on the map indicate the position of 168 georeferenced irrigation systems.

The survey targeted representatives from each WUA. Data gathering was conducted *on-site* using the open-source data collection and survey tool Kobo Toolbox (*www.kobotoolbox.org*). Kobo Toolbox is a globally used platform for data collection, management, and visualization, facilitating offline data collection even in remote locations with limited connectivity. The surveys included an extensive questionnaire of 400 questions covering 11 topics, including general information about location, water use, infrastructure, operational aspects, socio-administrative management, and prevailing irrigation practices.

## 2.3 Classification of irrigation systems

To understand the main issues encountered by users of irrigation systems, we classified these systems into four groups according to the size of the irrigated area. Since the irrigated areas can vary between 0.5 ha and 2400 ha, this classification allowed the analysis of comparable systems, to assess their primary challenges and strengths. The irrigation systems were categorized as follows: a) Micro Systems, with an irrigated area of less than 10 hectares; b) Small Systems, with an area from 10 to 100 hectares; c) Medium Systems, with 100 to 500 hectares; and d) Large Systems, with an irrigated area exceeding 500 hectares.

We analyzed various aspects related to irrigation and examined the role of WUAs to provide a comprehensive overview of the sustainability of irrigation systems. This analysis included assessing water quality perception, water allocation, water distribution, water sources for intake, infrastructural issues within the irrigation systems, role of the operator, water fees, water rights, and the establishment of boards, among others. By comparing these aspects, we identified key areas that require attention.

Based on the survey responses, we discussed the main challenges faced by these systems. This study represents a first attempt to understand the current state of irrigation systems, and assess their sustainability.

### 2.4 Water allocation and dependence on agricultural activities

Some of the most pressing questions are related to the differences in water distribution, basically the number of users/farmers that have water rights and the overall water allocated to the systems. These issues arise from the fact that farm sizes can vary widely from very small plots to big farms; thus, it is unknown the real number of water rights holders in each irrigation system and whether there is a relationship between them and total water allocation. We used scatter plots to examine the relationship between the number of users and the water allocated based on their irrigated area.

To evaluate potential differences in irrigated area per user and allocated discharge per user across irrigation system size categories, we employed the non-parametric Kruskal-Wallis test. This approach was chosen because the data violated assumptions of normality (Shapiro-Wilk test, $p < 0{,}001$) and homogeneity of variances (Levene's test, $p < 0{,}001$). Post-hoc pairwise comparisons using Dunn's test with Bonferroni correction were performed to identify specific group differences, with statistical significance set at $\alpha = 0{,}05$. Additionally, we explored potential associations among key variables (irrigated area, number of users, water rights cost, water fees) using Spearman's rank correlation.

Another frequent question is how agricultural and livestock production in these systems contribute to the farmers' subsistence. To investigate the reliance of farmers on these productive activities for their livelihoods, we employed boxplots. This approach enabled us to visually depict the variability and extent of dependence on agriculture and livestock across different irrigation systems.

### 2.5 Comparison of socioeconomic, water resources, governance and users' factors among irrigation systems

We extracted the data from the surveys to study the characteristics and factors that influence the functionality of WUAs and the sustainability of irrigation systems, as outlined in the United Nations World Water Development Report 2023. Table 1 provides an overview and summary of the information extracted from the surveys, which were analyzed and compared for each category of irrigation system (i.e., micro, small, medium, and large). This analysis allows to identify the most urgent priorities for addressing issues surrounding the sustainability of irrigation systems, with in turn affects food and water security.

## 3 Results and Discussion

### 3.1 Water allocation and dependence on agricultural activities

Discharge from streams and rivers is the main source of water for irrigation (71 % of systems), followed by water springs (26 %), lakes (2 %) and ground water (1 %). The most common systems are communal (90 %), followed by private systems (7 %) and public (3 %). Most irrigation systems are operated by WUAs (78 %) locally called "Juntas de riego", which are non-profit community organizations tasked with providing irrigation and drainage services according to Article 48 of the Regulation of the Water Resources and Water Use Law, 2015 (Correa, 2015). WUAs coordinate the administration, operation, and maintenance of the irrigation systems. According to the classification of irrigation systems, 62 medium systems have the highest total number of users (8433). Small systems also have an important number of users (121 systems with 6781 users). On the other hand, only 11 large irrigation systems serve a substantial number of users (5348). Conversely, there are 41 micro systems which 1,468 users, indicating the prevalence of smallholdings.

**Table 1.** Factors affecting the sustainability of irrigation systems. Modified from United Nations World Water Development Report 2023.

| Factor | Information |
|---|---|
| Socioeconomic | Main crops, Production for self-consumption, Primary productive activity, Target markets for sale, Added-value in products, Marketing challenges, Production losses associated with irrigation practices, Affiliation to rural producers' organizations, Harvest and transportation losses. |
| Water resources | Irrigation systems located above 3000 m a.s.l, Flow rates, Irrigation method, Reservoir systems, Irrigation infrastructure status, Water conveyance, Maintenance frequency, Water quality, Reduction in water flows, Increased frequency of droughts/floods, Water shifts in dry seasons. |
| Governance | Water usage fees, Water distribution rules, funding for restoration projects, Type of organization, Legal status, Water rights, Support and cooperation, Regulations. |
| Users | Problems in the irrigation system, Operation and maintenance of the system, Training of operators, Training of other users, Social capital, administrative, managerial and accounting skills. |

We found an unequal water distribution among users, regardless of their irrigated area. Indeed, the large scatter in Figure 2 illustrates a lack of relationship between the irrigated area per user and the allocated water discharge (l/s) per user. This disparity is evident across all system categories, where the number of users varies disproportionately for the same water flow and the allocated flow varies significantly for the same number of users. Some systems exhibit a very low water allocation per hectare, rendering them unsustainable. This situation may have arisen due to various factors, such as an increase in the number of users after the system's construction, or inaccurate measurements during the water allocation study. Conversely, some systems have a higher than necessary allocated discharge. It is imperative for local authorities to oversee these systems to address the unequal water distribution among users. Continuous monitoring is essential to ensure the sustainability of the systems.

The Kruskal-Wallis test revealed significant differences in allocated discharge per user across irrigation system size categories ($\chi^2 = 40{,}43$, $df = 3$, $p < 0{,}001$). Post-hoc Dunn's tests (Bonferroni corrected) indicated that small and micro systems differed significantly from large and medium systems ($p < 0{,}05$), while no significant difference was found between large and medium systems ($p = 0{,}489$). Similarly, irrigated area per user varied significantly across system categories ($\chi^2 = 112{,}97$, $df = 3$, $p < 0{,}001$). Post-hoc comparisons showed that users in small and micro systems had significantly different irrigated areas compared to those in large and medium systems ($p < 0{,}001$), again with no significant difference between large and medium systems ($p = 0{,}9425$). These results indicate that irrigation system size is significantly associated with the per-user distribution of both irrigated area and allocated water, suggesting greater disparities in smaller systems.

Spearman correlation analyses among irrigated area, number of users, water rights cost, and water fees yielded only weak associations, indicating no strong linear relationships between these variables in this dataset.

The livelihood of farmers depends on agricultural and livestock activities to varying extents (Figure 3). Medium and large systems demonstrate a more direct reliance on productive activities, whereas small and micro systems exhibit wider variation. From the 235 irrigation systems analyzed, 151 (64%) relied on agricultural activities for more than 70% of their livelihoods. Specifically, this includes 43 medium systems with 5,167 users, 77 small systems with 3,785 users, 7 large systems encompassing 2,593 users, and 24 micro systems covering 952 users. This analysis underscores the significance of irrigated agriculture in the rural economies of mountainous regions. However, while these numerous small and micro systems are evidently crucial for local livelihoods, their overall productivity and long-term economic sustainability may be constrained by common challenges. Research by Berhe et al. (2022) on small-scale irrigation in Ethiopia, for instance, reveals that many such schemes operate below their design potential due to persistent issues in operation, maintenance, and the capacity of local managing institutions, often exacerbated by limited financial resources.

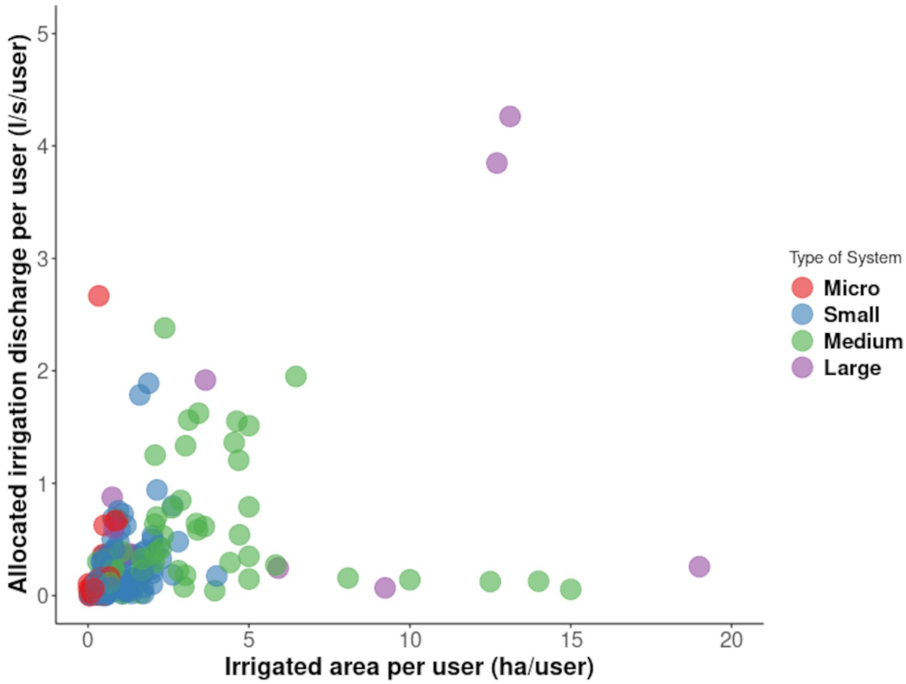


**Figure 2.** Relationship between irrigated area (ha/user) and allocated water discharge (l/s/user) for each category of irrigation system.

This suggests that the potential of the micro and small systems in our study area to substantially enhance agricultural output and secure lasting economic benefits for users could be undermined if these prevalent operational and financial difficulties, also highlighted by Berhe et al. (2022), are not effectively addressed. Conversely, only 10% of irrigation systems depend on agricultural and livestock activities for 25% or less of their livelihoods. This low dependence may be attributed to the presence of peri-urban systems and migration of people to bigger cities, potentially leading to land abandonment and jeopardizing food security. Additional factors, such as the younger population's preference for non-agricultural employment, may also contribute to this trend. Many users or farmers may seek alternative means of subsistence by working in nearby urban areas. In order to understand these issues and the actual conditions of irrigation systems, it is crucial to continue data collection, emphasizing additional relevant, complementary, and critical information to support informed decision-making.

## 3.2 Comparison of socioeconomic, water resources, governance and users' factors among irrigation systems

Socioeconomic factors influencing irrigation systems are described in Table 2. The primary crops across all systems include pastures, intercropped maize beans, and vegetables. Except for medium systems, the percentage of land used for pastures surpasses that for crops. This could be explained by the fact that most of the production area in Azuay is intended for natural and cultivated pastures (GPA, 2018). Livestock activities, particularly dairy production, hold significant economic importance in Azuay, accounting for 8 % of the national milk production (GPA, 2018). However, dairy production yields are low, indicating the need to strengthen management practices to make it more competitive and equitable for producers.

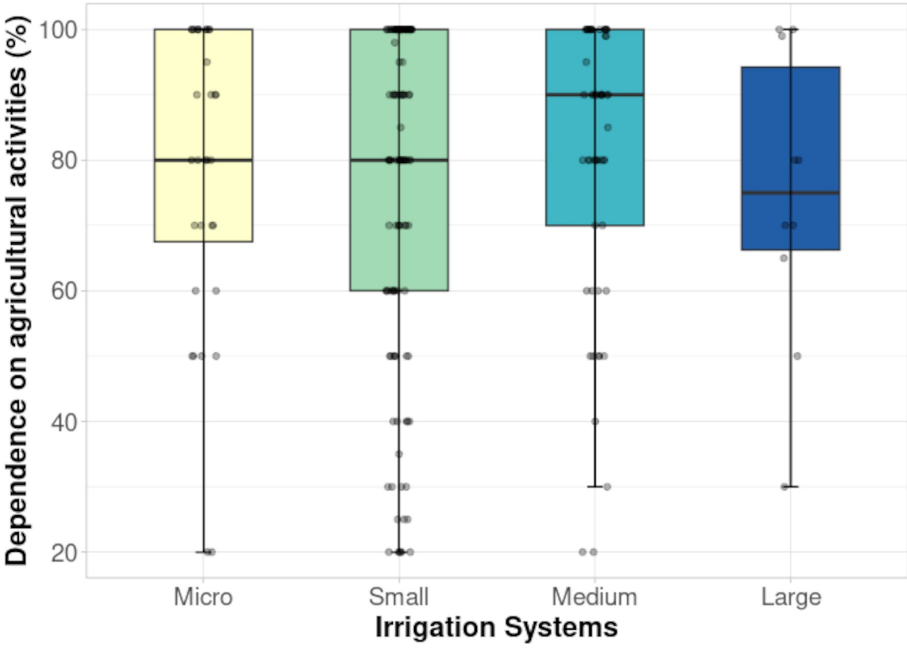


**Figure 3.** Livelihood dependence on agricultural and livestock activities for each category of irrigation systems.

Farmers stated that the main factor contributing to production losses is the lack of irrigation water. In small systems, there is a concern about the misuse of water, which worsens the situation. People from medium systems perceive that droughts further affect production. Effective water management practices can mitigate the risks associated with water scarcity and distribution inequalities, which are critical for sustaining agricultural livelihoods and rural economies. Decision-makers need to prioritize interventions that enhance irrigation infrastructure, promote sustainable water use, and support the overall resilience of the agricultural sector.

Productive activities are primarily subsistence-level, even among irrigation systems with larger irrigated areas (i.e., small, medium, and large), with most production intended for self-consumption. This situation contributes to increased rural migration and challenges to local food security. On the other hand, the prima-ry challenge faced by all irrigation systems is securing a fair price for their products to ensure profitability. A key issue is the absence of clear policies and incentives for supporting small-scale farmers in obtaining fair prices for their products. Middlemen frequently purchase produce at significantly low prices, capturing a disproportionate share of the profits. The lack of fair trade exacerbates the situation, compelling producers to accept reduced prices to avoid product loss, thus perpetuating the advantageous position of middlemen in the market chain. This finding strongly aligns with research by Rebaï (2017), who identifies the economic vulnerability of family farmers in Azuay as stemming principally from their lack of market access and subordination to intermediary actors, severely limiting their commercial integration. Indeed, the persistence of such market-related challenges for small agricultural associations, despite policies aiming to support them, is also highlighted by Gómez-Ceballos et al. (2021) in Ecuador, where significant difficulties in how markets operate for these associations were found to hinder their economic progress.

**Table 2.** Socioeconomic factors influencing sustainability for each category of Irrigation Systems. Multiple responses allowed; totals may exceed 100 %.

| **Socioeconomic factors** | **Micro systems (<10 ha)** | **Small systems (10-100 ha)** | **Medium systems (100-500 ha)** | **Large systems (>500 ha)** |
|---|---|---|---|---|
| Main Crop % | Pasture (83 %) | Pasture (97 %) | Pasture (84 %) | Pasture (100 %) |
| Second Crop % | Vegetables (81 %) | Intercrop maize/ beans (83 %) | Intercrop maize/ beans (84 %) | Intercrop maize/ beans (73 %) |
| Production for self-consumption | Yes (73 %) | Yes (65 %) | Yes (60 %) | Yes (55 %) |
| Main Target market$^{mo}$ | Cantonal (76 %) | Cantonal (69 %) | Cantonal (84 %) | Cantonal (82 %) |
| Added value in products | No (76 %) | No (74 %) | No (81 %) | No (64 %) |
| Marketing challenges$^{mo}$ | Low prices (73 %) | Low prices (82 %) | Middlemen (81 %) | Low Prices / Middlemen (64 % each) |
| Primary production loss factor$^{mo}$ | Lack of water (71 %) | Lack of water (58 %) | Lack of water (57 %) | Lack of water (73 %) |
| Affiliated to Producer Organizations | 7 % | 13 % | 13 % | 18 % |
| Harvesting and transportation losses | Yes (10 %) | Yes (16 %) | Yes (21 %) | Yes (9 %) |

$^{mo}$ = Respondents could select multiple options for factors marked (mo) in the original survey; percentages reflect selection frequency for each option. “Main” indicates the most frequent response.

Another prevalent issue is the lack of organization and opportunities to participate in productive associations, which have the potential to facilitate direct market access. This observed weakness resonates with Rebaï (2017) central argument that strengthening farmer organizations is fundamental for overcoming market access barriers and improving rural-urban linkages. According to Rebaï (2017), reinforcing these organizations is crucial not only for improving economic integration and negotiation power with public authorities but also for potentially fostering collaboration among farmers to enhance productive systems and natural resource management (Ostrom, 1990). Notably, the Provincial Government of Azuay has made efforts to establish direct markets, aiming to benefit producers and ensure equitable remuneration for their endeavors. This approach not only enhances long-term profitability for farmers but also ensures a consistent supply of high-quality products for consumers.

Table 3 presents factors related to water resources. Users of irrigation systems state that the status of irrigation infrastructure ranges from good to regular. However, in larger systems, infrastructure is often rated as bad to regular, indicating a need for increased maintenance expenses. Larger systems report the need of more frequent maintenance, approximately every month, in contrast to smaller systems, where maintenance is more sporadic (6 to 12 months). The frequent need for maintenance (mainly for sediment cleaning and repairing leaks) underscores the problems of large systems. These insights have important management implications, suggesting that large systems face additional costs and that targeted actions should be taken to understand and address specific issues in these systems.

While over 55 % of farmers perceive the water quality from their irrigation systems as good, cases of medium quality are often associated with organic residues from agriculture and livestock near water sources. Regarding farmers’ perception of water flow variations, a significant decrease in flows is reported, particularly in larger irrigation systems. This reduction in flows is linked to the expansion of the agricultural frontier and, in some cases, the clearing of native vegetation and primary forests to grow pasture for livestock breeding. These impacts mainly affect the lower part of the catchment area, with users in this zone experiencing greater impacts compared to those in the headwaters. In any case, a degrading water quality and a reduction in discharge could negatively impact food security and have to be taken in consideration by policy makers and water managers.

Indeed, the degradation of resources like water and soil can ultimately undermine the viability of agricultural systems and livelihoods. This aligns with findings by López-Carr et al. (2017), who identified soil degradation as a key driver of out-migration from rural communities in Guatemala, suggesting a critical link between resource health and the potential for population displacement or system abandonment if such degradation is not addressed. Despite the high altitude (over 3000 m.a.s.l), few irrigation systems still function, particularly for micro and small-scale farming, where irrigation is inappropriate due to the conservation status of these zones.

**Table 3.** Assessment of Water Resources factors influencing sustainability in four types of Irrigation Systems. Multiple responses allowed; totals may exceed 100 %.

| Water Resources factors | Micro systems (<10 ha) | Small systems (10-100 ha) | Medium systems (100-500 ha) | Large systems (>500 ha) |
|---|---|---|---|---|
| Irrigation systems >3000 m a.s.l. | 29 % | 26 % | 16 % | 9 % |
| Main irrigation method[mo] | Sprinkler (68 %) | Sprinkler (74 %) | Gravity-fed irrigation (69 %) | Sprinkler /gravity-fed irrigation (64 % each) |
| Systems with reservoirs | 54 % | 46 % | 37 % | 27 % |
| Infrastructure status | Good (34 %) | Good (31 %) | Good (24 %) | Regular (27 %) |
| Main Maintenance frequency | 6 months (27 %) | 6 months (30 %) | 6 months (36 %) | 6 months / 1 month (36 % each) |
| Water quality perception | Good (66 %) | Good (60 %) | Good (68 %) | Good (55 %) |
| Sediments in irrigation water | Yes (56 %) | Yes (60 %) | Yes (52 %) | Yes (82 %) |
| Perception of declining water flows | Yes (51 %) | Yes (65 %) | Yes (74 %) | Yes (82 %) |
| Perceived Main Cause Flow Reduction | Native forest clearing (71 %) | Native forest clearing (79 %) | Native forest clearing (82 %) | Native forest clearing (46 %) |
| Change of water shifts during dry seasons | No (61 %) | No (82 %) | No (76 %) | No (82 %) |
| Increased droughts in the past decade | 51 % | 63 % | 76 % | 82 % |

[mo] = Respondents could select multiple options for factors marked (mo) in the original survey; percentages reflect selection frequency for each option. "Main" indicates the most frequent response.

Another critical concern is the continued reliance on traditional irrigation methods (i.e., not pressurized) that contribute to significant water losses. Moreover, most irrigation systems do not have dams or reservoirs to mitigate the impact of drought, rendering them vulnerable. This susceptibility is corroborated by users' perceptions of increased droughts lately. Despite their awareness of drier seasons, no policies or adaptation strategies have been implemented. This situation highlights a broader challenge in water governance. Research in Central Asia by Abdullaev et al. (2025), for example, underscores that addressing water scarcity effectively requires more than pursuing efficiency alone; it demands a shift in governance. They argue that true adaptation necessitates dismantling significant existing barriers and fundamentally reorienting water governance approaches to build robust, long-term preparedness for an uncertain future. Complementing this view, Sirimewan et al. (2021b) emphasize, from a socio-technical perspective, that while adopting improved technologies like sprinkler or drip irrigation is vital for sustainable water use, these technical solutions cannot succeed in isolation. Their research in Sri Lanka highlights that the successful uptake of such efficient practices also depends critically on supportive social, management, and regulatory environments.

Governance issues in the irrigation systems are examined in Table 4. The absence of clear regulations is evident within irrigation systems, particularly concerning water-use fees, water distribution and water-rights acquisition. It was surprising to find that a significant number of WUAs lack legal recognition (29 – 44 %). This lack of formal status presents several drawbacks. Without legal recognition, WUAs face challenges in managing their systems effectively, securing funds for system improvements, and engaging with NGOs and other external organizations. Legal recognition is crucial for WUAs to operate with full authority, access financial resources, and collaborate with stakeholders to enhance the sustainability and efficiency of their irrigation systems.

Another unexpected finding is that in a large number of irrigation systems, farmers do not pay water fees. According to Ecuadorian law specified in Article 116 of Regulation of the Water Resources and Water Use Law (Correa, 2015), all water users are mandated to pay fees for water usage. This finding highlights the weak enforcement of the regulations. While over 40 % of systems do not impose fees at all, it is remarkable that large systems comply with this regulation. The implementation of these fees aims to promote the independence of WUAs in managing irrigation systems and reducing their dependency on external institutions for infrastructure investment and maintenance. This independence ultimately aims to enhance the efficiency of water distribution.

A shift towards participatory governance is vital, incorporating principles of fair pricing designed to cover comprehensive costs like planning, climate adaptation, and maintenance (WWAP, 2019). Integral to the success of such governance, however, is the active prevention of management inefficiencies, which are identified as key factors that can increase system vulnerability and hinder proper water management (Pacheco-Peña et al., 2023).

Moreover, more than 70 % of irrigation systems operate independently, without collaborative arrangements with external institutions. This independence, combined with non-compliance with water use fees, often hinders effective irrigation management. While a significant majority of WUAs express a willingness to engage with external institutions, there are noticeable instances where WUAs are hesitant to participate in collaborative efforts. The reluctance observed in some WUAs is a critical factor for consideration by decision-makers. To navigate this, decision-makers should focus on two key actions: 1) actively and promptly supporting WUAs that are willing to collaborate, including fostering opportunities for NGOs, and 2) initiating a dialogue process designed to understand and address the reservations of those WUAs that are hesitant. Academia plays a pivotal role in addressing these issues. When tackling these governance challenges, decision-makers essentially choose from a spectrum of action, as FAO (2024) suggests. Options range from working within existing power structures to implementing more transformative changes that alter the influence of different actors (e.g., strengthening WUAs). This choice between incremental adjustment and structural reform is critical, affecting the feasibility and ultimate impact of governance improvements.

We found that farmers can acquire water use rights through various means. In addition to payments, water rights can be obtained by contributing labor to construct irrigation systems or through inheritance. In larger systems, participation in the legalization of irrigation systems serves as an alternative route for obtaining water rights. Additionally, the tying of water rights to either land or individuals leads to inequitable payments, as individuals' rights can be utilized across multiple plots. Moreover, the water allocated to users' plots does not consider the plot area, potentially resulting in either insufficient or excessive water for agricultural and livestock activities. Addressing these complexities effectively likely requires a multi-criteria analysis within a co-management framework, involving all actors to develop allocation and rights systems that promote both sustainability and efficiency (Rivera, 2016), acknowledging water's role in food sovereignty and local economies (Pacheco-Peña et al., 2023).

**Table 4.** Assessment of Governance factors influencing sustainability in four types of Irrigation Systems. Multiple responses allowed; totals may exceed 100 %.

| Governance factors | Micro systems (<10 ha) | Small systems (10-100 ha) | Medium systems (100-500 ha) | Large systems (>500 ha) |
|---|---|---|---|---|
| WUAs paying water usage fees | 59 % | 65 % | 58 % | 73 % |
| Main Water distribution rule | Surface-independent water distribution (49 %) | Surface-independent water distribution (46 %) | Proportional to the surface area (42 %) | Proportional to the surface area (46 %) |
| WUAs received external catchment funding | No (61 %) | No (74 %) | No (82 %) | No (64 %) |
| WUAs have legal recognition | 56 % | 60 % | 71 % | 64 % |
| Main Organization Assisting Construction | Regional government (49 %) | Regional government (63 %) | Regional government (63 %) | Regional government (64 %) |
| Main Definition of water rights | Earned (participation in the construction) (54 %) | Purchased (59 %) | Purchased (60 %) | Purchased (55 %) |
| Main Type of water rights | Tied to land parcel (49 %) | Tied to land parcel (55 %) | Tied to land parcel (73 %) | Tied to land parcel (73 %) |
| WUA Willingness to Engage Externally | High (66 %) | High (69 %) | High (69 %) | High (82 %) |
| Internal Operation Guidelines Exist | Yes (61 %) | Yes (72 %) | Yes (68 %) | Yes (82 %) |
| Compliance with internal rules | Partially (37 %) | Yes (38 %) | Yes (47 %) | Yes (73 %) |
| Main Prohibited Social Practice[mo] | Water sharing (88 %) | Exchange shifts (98 %) | Exchange shifts (82 %) | Exchange shifts (100 %) |

[mo] = Respondents could select multiple options for factors marked (mo) in the original survey; percentages reflect selection frequency for each option. "Main" indicates the most frequent response.

Despite the presence of internal regulations and internal operation guidelines for the irrigation system, especially in micro and small systems, compliance is often lacking, indicating a deficiency in governance necessary for effective and sustainable management. This lack of proper operation leads to disorganization, particularly in water distribution during water shifts.

Although users within irrigation systems do not exchange water shifts for products, work, or loans, the lack of clear regulations allows a significant percentage of users to access water from others. Water division is often decided by users, particularly in larger systems. This is an important aspect of governance and technical management. Further studies are needed to analyze how these practices can be improved to enhance irrigation management.

Table 5 lists the various user-related parameters. Users in all irrigation systems acknowledge that the main issue is infrastructural problems, which become more pronounced as the irrigation system grows larger owing to sediment accumulation and the high need for constant maintenance. A significant concern is the general absence of trained operators for irrigation systems. When someone is designated for this role, they often lack proper training and rely instead on limited experience. While some users have received training in areas such as technical management; socio-organizational aspects; and administration, operation, and maintenance, very few users in micro, small, and medium systems have accessed this training. In contrast, larger systems seem to have better organizational structures; however, nearly half of them do not have an irrigation operator, and of those that do, more than half are not well trained to perform the task.

Irrigation schedules often occur during both the day and night. This can lead to significant issues in terms of correct water use, particularly when irrigation occurs at night. Water losses are higher in systems where irrigation is not pressurized and lacks automatic control technology. Moreover, they can trigger mass movements and landslides. It is crucial to address these issues by implementing advanced irrigation technologies that allow for precise water control. In addition, establishing clear guidelines and providing training on optimal irrigation practices can help mitigate water waste and prevent landslides. Decision-makers should prioritize the adoption of automated irrigation systems and support initiatives that enhance the technical knowledge of farmers and irrigation system operators.

However, a key strength across all irrigation systems is the social capital that facilitates collective work through mingas for the common good. Indeed, mingas are the primary method for system maintenance and other activities. This reliance on collective action, rooted in local norms of reciprocity and trust, is crucial for the management and adaptation of Andean irrigation systems, a finding supported by research in both the Venezuelan Andes (Leroy et al., 2022) and the Ecuadorian Highlands (Hoogesteger, 2015).

**Table 5.** Assessment of "Users" factors influencing sustainability in four types of Irrigation Systems. Multiple responses allowed; totals may exceed 100 %.

| "Users" factors | Micro systems (<10 ha) | Small systems (10-100 ha) | Medium systems (100-500 ha) | Large systems (>500 ha) |
|---|---|---|---|---|
| Primary system concern | Infrastructure (42 %) | Infrastructure (63 %) | Infrastructure (58 %) | Infrastructure (91 %) |
| Primary solution considered | Infrastructure improvement (59 %) | Infrastructure improvement (68 %) | Infrastructure improvement (66 %) | Infrastructure improvement (82 %) |
| Main Current operation and maintenance issue (mo) | Damage main irrigation canal (59 %) | Damage main irrigation canal (80 %) | Damage main irrigation channel (84 %) | Damage main irrigation canal (91 %) |
| Irrigation Schedule (Variable Day/Night) | -63 % | -72 % | -87 % | -100 % |
| System has an operator | No (78 %) | No (82 %) | No (65 %) | No (55 %) |
| Operator considered qualified | Yes (50 %) | Yes (73 %) | Yes (80 %) | Yes (60 %) |
| Operator received training | No (100 %) | No (64 %) | No (65 %) | No (40 %) |
| Other users trained to operate | No (68 %) | No (80 %) | No (71 %) | No (64 %) |
| Main user training topic received | Administration, operation, and maintenance (17 %) | Administration, operation, and maintenance (15 %) | Administration, operation, and maintenance (8 %) | Technical management of the system (36 %) |
| Main maintenance method | Collective efforts by users ("minga") (90 %) | Collective efforts by users ("minga") (94 %) | Collective efforts by users ("minga") (89 %) | Collective efforts by users ("minga") (100 %) |

mo = Respondents could select multiple options for factors marked (mo) in the original survey; percentages reflect selection frequency for each option. "Main" indicates the most frequent response.
Minga: Word of Quechua origin that refers to a collective effort for the common good.

### 3.3 Limitations of the study

This study draws upon data from a 2022 survey administered by the Provincial Government of Azuay. While offering valuable regional insights into irrigation systems, this data source carries inherent limitations that should be indicated.

The primary limitation stems from the data collection method. Information was gathered from representatives of WUAs. Although these individuals are generally knowledgeable about their respective systems, their perspectives may not fully encompass the diverse experiences and views of all individual water users. Furthermore, responses could be influenced by their leadership roles, potentially introducing a degree of subjectivity. Consequently, data regarding specific quantitative or potentially sensitive topics, such as individual plot productivity or earnings, might reflect estimates rather than precise figures, as representatives may not possess or share complete information for all members.

Furthermore, the selection of WUA representatives as respondents might introduce a significant gender bias. If these representatives were predominantly male –a common scenario in such organizations– the survey would inherently fail to capture women's perspectives on crucial aspects like participation in decision-making roles or their views on the effectiveness and equity of irrigation system management. Consequently, the results may not fully represent the experiences and viewpoints of the entire user community, particularly concerning gender-specific challenges or priorities.

Despite these constraints, the survey data provides a valuable assessment of the status of irrigation governance and infrastructure in Azuay. The findings successfully identify key challenges and priority areas, offering essential guidance for targeted future research and policy development. This study also highlights the need for subsequent, more systematic data collection, potentially incorporating stratified sampling at the individual user level and methods designed to capture diverse perspectives, to support robust and equitable long-term water resource management.

## 4 Conclusions

After a comprehensive analysis of the comparative factors among irrigation systems, several critical issues regarding their management and sustainability were identified, requiring continued monitoring and further investigation. Effective governance has emerged as the main concern, as it influences system administration, funding acquisition, fee collection, and long-term stability. Without robust governance structures, these systems are struggling to function efficiently. Equitable water distribution is another significant issue; authorities must update and regulate water delivery to ensure that systems receive the technically required amount from available sources, adopting a technical-environmental approach to water allocation.

Lack of governance further hinders the sustainability of the system. Systems without governance cannot raise the necessary funds for operation and maintenance, establish a technical team for the day-to-day management and advisory roles, create operating rules, or achieve a legal status. The role of the regional government and the Ministry of Agriculture is crucial in supporting these systems by providing ongoing training for operators, legal constitutional support, and access to markets.

Moreover, in a significant number of systems, agricultural production is no longer the primary source of income, thereby putting food security at risk. Addressing this issue requires targeted intervention by public institutions. The system's vulnerability is also evident: if fee collection fails, the system stops operating; if the operator is unavailable, there is no replacement; without operating rules, the procedures are unclear; and in droughts, water scarcity becomes critical.

Systematic monitoring is necessary as the conditions may change. Annual monitoring will help track system evolution, analyze the impact of policies and interventions, and assess the effects of climate and socioeconomic conditions such as migration. Developing performance indicators suited to local realities is essential. By addressing these key areas, decision-makers can take targeted actions to improve the management and sustainability of irrigation systems, ultimately supporting resilience and productivity in the agricultural sector.

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## Authors' contributions

G.B.: Conceptualization, methodology, formal analysis, data curation, writing-original draft preparation. R.C.: Conceptualization, methodology, funding acquisition, writing-review and editing.

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Dynamics of Water and the Páramos

# Community management and sustainability in Andean irrigation systems through indicators of efficient water use in agriculture

## Gestión comunitaria y sostenibilidad en sistemas de riego andinos mediante indicadores de uso eficiente del agua en la agricultura

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**Abstract**

Globally, increasing competition for water and the effects of climate change have heightened the need to assess the sustainability of irrigation systems, particularly in strategic ecosystems such as the Andean páramos. However, there is a gap in methodological tools that integrate indicators adapted to community-based contexts and aligned with global frameworks such as the Sustainable Development Goals (SDGs) and the Principles for Responsible Investment in Agriculture and Food Systems (RAI Principles). This study aims to define indicators for evaluating the sustainability of community-managed irrigation systems in Ecuador. The MESMIS framework and the Delphi technique were applied using a participatory approach that involved community leaders, technicians, academics, and students. A total of 31 indicators were defined, organized into seven attributes and five dimensions (environmental, social, economic, political, and technological), and aligned with nine SDGs and seven RAI Principles. The results reveal critical issues related to water use efficiency, governance, equity, and system resilience. The proposed framework enables a comprehensive and context-specific evaluation of irrigation systems and provides a practical tool for public policy design. In conclusion, this research helps bridge the existing methodological gap and reinforces the role of community irrigation systems as key pillars for sustainable and resilient agriculture.

***Keywords***: MESMIS, páramos, sustainable irrigation, community water management, sustainability indicators.

**Resumen**

A nivel global, la creciente competencia por el agua y los efectos del cambio climático han acentuado la necesidad de evaluar la sostenibilidad de los sistemas de riego, especialmente en ecosistemas estratégicos como los páramos andinos. Sin embargo, existe un vacío en herramientas metodológicas que integren indicadores adaptados a contextos comunitarios y alineados con marcos globales como los Objetivos de Desarrollo Sostenible (ODS) y los Principios de Inversión Responsable en Agricultura (CSA-IRA). Esta investigación tiene como objetivo definir los indicadores para evaluar la sostenibilidad de sistemas de riego comunitarios en Ecuador. Se consideró la metodología MESMIS y la técnica Delphi mediante un enfoque participativo que incluyó líderes comunitarios, técnicos, académicos y estudiantes. Se definieron 31 indicadores, organizados en siete atributos y cinco dimensiones (ambiental, social, económica, política y tecnológica), articulados con nueve ODS y siete Principios CSA-IRA. Los resultados evidencian puntos críticos en la eficiencia hídrica, gobernanza, equidad y resiliencia de los sistemas. La propuesta permite una evaluación integral y contextualizada de los sistemas de riego, y ofrece una herramienta práctica para el diseño de políticas públicas. En conclusión, se contribuye a cerrar el vacío metodológico existente y se fortalece el rol de los sistemas de riego comunitarios como pilares para una agricultura sostenible y resiliente.

***Palabras clave***: MESMIS, páramos, riego sustentable, gestión comunitaria del agua, Indicadores de sostenibilidad.

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# 1 Introduction

The sustainability paradigm has broadened the analytical framework of natural systems by integrating economic, environmental, and social dimensions applicable to agricultural production (González et al., 2006; Guo and Yu, 2022). However, Talukder et al. (2020) question whether sustainable agricultural systems can truly ensure food security, particularly in low-income countries. These authors argue that achieving this goal requires a "sustainable intensification" of resource use, such as water, through advanced technologies that minimize or eliminate adverse environmental impacts, such as rainwater harvesting (Cachipuendo et al., 2024). In this context, irrigation, considered a key system in agriculture, must be evaluated from a sustainability perspective.

Irrigation plays a fundamental role in water and food security (Darzi-Naftchali et al., 2020), and its sustainability should be analyzed in the context of climate change, taking into account factors such as natural resource conservation, technological innovation, and water use efficiency in agriculture (Velasco-Muñoz et al., 2018; Darzi-Naftchali et al., 2020). Irrigation is commonly regarded as a means of production that enhances water management in agriculture (Wang and Wu, 2018), as well as a socio-ecological-technical system that integrates physical, organizational, social, and natural components (Newman et al., 2011). Nonetheless, its management faces technical and financial challenges, especially in contexts where irrigation governance has been transferred from government agencies to farmer associations or private entities (Nagrah et al., 2016; Shalsi et al., 2022). This shift has led to uneven performance and limited practical outcomes (Araral, 2005).

For rural communities, irrigation is not merely an agricultural production tool (Brugnach et al., 2017), but a complex system where nature, community, and infrastructure converge (Cachipuendo Ulcuango et al., 2021). Its operation generates social, environmental, and economic interrelations (Fernald et al., 2012), which are structured within organizations that manage and operate irrigation systems according to each country's legal framework (Herrán et al., 2017). Given the climate crisis and population growth, it is increasingly necessary to boost food production in alignment with the Sustainable Development Goals (SDGs) and the Principles for Responsible Investment in Agriculture (CSA-IRA) (Jägermeyr et al., 2017).

Considering the sociocultural, environmental, political, and technological dynamics of irrigation systems, it is essential to identify integrative mechanisms that enable the analysis of critical points across their components in order to establish sustainable and efficient water use strategies.

In the Andes, agricultural water comes mainly from surface and groundwater sources fed by glaciers-which have decreased by 25% over the past 30 years due to climate change (Gallegos et al., 2018)-as well as from wetlands formed in the paramo ecosystem, which capture rain and fog that subsequently infiltrate the soil. However, paramo and high Andean forest ecosystems are losing their water retention capacity due to anthropogenic and climatic causes (Llambí et al., 2012). In response, irrigation organizations have implemented strategies such as pressurized irrigation, which have improved the resilience of farmers and communities (Cachipuendo, 2022). Therefore, the sustainability of these systems requires a comprehensive evaluation that considers social, environmental, economic, political, and technological dimensions (Chile and Ortiz, 2021).

Traditionally, the study of irrigation systems has followed a disciplinary approach, focusing on specific indicators such as physical water use efficiency at the plot level, economic performance, and environmental impacts (Cachipuendo Ulcuango, 2021). This fragmented approach does not comprehensively address system sustainability nor the interrelationship among its dimensions. Methodologies such as MESMIS (Framework for the Evaluation of Natural Resource Management Systems Incorporating Sustainability Indicators) offer tools based on systems thinking to assess sustainability dynamically, flexibly, and in ways adapted to local realities (Masera et al., 2000).

Evaluating the sustainability of a natural system entails identifying its physical, social, environmental, political, and economic components and analyzing their interactions through systemic models (Samian et al., 2015; Carmona et al., 2013). In the Andes, irrigation system evaluation is structured into three subsystems: nature, community, and land use (Cachipuendo Ulcuango, 2021). Each subsystem requires specific indicators, such as water availability and quality (Costa et al., 2022), the organizational capacity of communities (Turner et al., 2016), water use economic efficiency (Meng et al., 2022), and irrigation technology adoption (Laali et al., 2022). The use of frameworks like MESMIS allows for the integration of indicators and the simultaneous assessment of sustainability dimensions, including stakeholder participation (Sarandón, 2010; Franco et al., 2012). This approach has proven effective in Andean contexts for evaluating agroecosystems and livestock or agricultural production systems (Vallejo et al., 2020; Tongo and Soplín, 2022).

Globally, sustainability must align with the SDGs, which provide a framework for addressing common global challenges and developing policies tailored to local contexts (United Nations, 2015; Persson et al., 2016). Additionally, the CSA-IRA Principles aim to ensure responsible investment in agriculture and irrigation systems, although their non-binding nature poses challenges for producers when disputes arise with investors (Stephens, 2013).

In Ecuador, given the community-based organization managing irrigation systems, there is a need for tools to evaluate their sustainability while accounting for local realities without losing sight of global objectives. Therefore, the objective of this study is to define indicators for evaluating the sustainability of irrigation systems in Ecuador within the MESMIS methodological framework, taking into account their relationship with the SDGs and the CSA-IRA Principles.

# 2 Materials y Methods

## 2.1 Study Area

This research was conducted in Ecuador, located on the northwestern coast of South America. The country is crossed by the equatorial line and has altitudes ranging from 0 to 6.263 meters above sea level. The largest irrigated areas are primarily found in the provinces of Guayas (260 000 ha), Chimborazo (124000 ha), and Pichincha (107000 ha). Of the total irrigated land, 22% corresponds to public irrigation systems, 40% to community-managed systems, and 38% to private systems (Gaybor, 2019). There are 3.425 community-managed irrigation systems distributed mostly across the Ecuadorian Andes. These systems have been constructed by users through communal labor (in spanish "mingas") in steep mountainous regions. They serve plots averaging less than one hectare and are operated by smallholder farmers engaged in subsistence agriculture, often under precarious conditions that compel them to seek additional employment outside their agricultural production units (UPAs) (Gaybor, 2019).

Considering the types of inter-community, community, collective, and public irrigation systems (Cachipuendo Ulcuango et al., 2021), the study identified three inter-community irrigation systems in the provinces of Tungurahua, Pichincha, and Cotopaxi; twelve community-managed systems—one in Carchi, two in Imbabura, three in Pichincha, two in Cotopaxi, two in Tungurahua, and two in Chimborazo; four collective or associated irrigation systems in Pichincha; and one public irrigation system in Carchi.

## 2.2 Scope of the Research and Indicator Definition

The research used a descriptive and correlational scope and was developed using the MESMIS methodology, complemented by the Delphi Panel technique (expert consultation). This methodological combination enabled the identification and definition of a set of 31 indicators aimed at evaluating the sustainability of irrigation systems. The process was structured in four stages (Figure 1).

(1) Definition of the irrigation system functioning model. This stage was carried out through six focus groups involving 36 community leaders. A structured guide with ten questions on the management and operation of the irrigation system was used as the data collection instrument.

(2) Identification of diagnostic criteria and critical points of the irrigation system, following the guidelines of the MESMIS methodology (Masera et al., 2000; Astier et al., 2008). Semi-structured interviews and surveys were conducted with a group of 18 key informants: 6

technicians, 4 academics, and 8 graduate students. This process allowed for the delimitation of the main criteria for assessing the sustainability of irrigation systems.

(3) Definition of indicators. In this stage, a Delphi Panel was formed with 12 experts in irrigation system management and governance, representing government institutions, universities, and the private sector. The process included two rounds of consultation: the first focused on the conceptual validity and relevance of the proposed indicators, and the second aimed at determining the degree of influence of each indicator on system sustainability.

(4) Analysis of the indicators in relation to the SDGs and CSA-IRA Principles. A semantic analysis was conducted to establish the correspondence and alignment of the indicators with these international reference frameworks. This analysis revealed that the defined indicators for evaluating community-managed irrigation systems align with 10 Sustainable Development Goals and 7 CSA-IRA Principles (Table 1), underscoring the potential of functioning irrigation systems to contribute to the achievement of global sustainable development objectives.

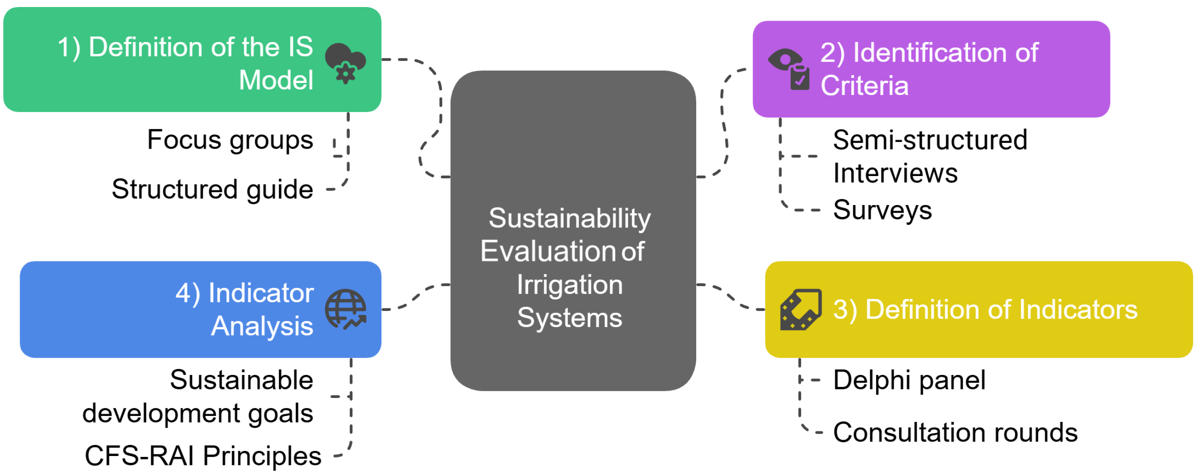


**Figure 1.** Stages and Methods for the Construction of Indicators for the Evaluation of Irrigation.

## 3 Results

The results of this research reflect a comprehensive methodological approach for evaluating the sustainability of community-managed irrigation systems in the Ecuadorian Andean context. Through the application of the MESMIS framework and the Delphi technique, a diagnostic model was developed to identify the key components of the irrigation system-nature, community, and infrastructure-as well as the main critical points affecting its performance. A total of 31 indicators were defined, aligned with seven sustainability attributes and five dimensions (environmental, social, economic, technological, and political), and articulated with nine Sustainable Development Goals (SDGs) and seven CSA-IRA Principles. The findings reveal structural, organizational, and technical limitations that influence water efficiency, equity in distribution, governance, and the resilience of these systems, providing a solid foundation for the design of public policies and community strengthening strategies.

### 3.1 Functioning Model of the Irrigation System

The functioning model of a community-managed irrigation system is based on three fundamental elements: nature, community, and infrastructure (Figure 2). The nature subsystem includes the water inputs into the system, which depend on exogenous factors such as temperature, precipitation, wind, and solar radiation. In the case of the Ecuadorian highlands (Sierra), the main water sources are the permanent Andean snowcaps and the paramos, whose high Andean forest ecosystems capture water and release it to lower elevations through springs or runoff (Llambí et al., 2012). However, anthropogenic activities such as the expansion of the agricultural frontier, burning of paramo vegetation, and grazing reduce the aquifer recharge capacity, constituting significant internal factors that affect the system.

The community subsystem encompasses human actions at the individual, communal, or collective levels that impact the physical, economic, social, and environmental efficiency of agricultural water use. Water management in irrigation systems involves specific procedures for the access, conveyance, storage, distribution, and efficient application of water, aimed at minimizing waste. Four key factors were identified within this subsystem: i) Knowledge- Refers to the users' understanding, individually or collectively, of the optimal irrigation timing based on crop type; ii) Social Participation– Includes user involvement in community activities, decision-making processes, and training or capacity-building programs; iii) Institutional-Pertains to the organization's ability to operate, maintain, and manage the system, including aspects such as water scheduling, leadership rotation, and the type of organization; iv) Economic- Refers to the financial capacity of users and organizations to access funding, maintain and upgrade the system, as well as the existence of policies that support these activities.

The infrastructure subsystem comprises the physical components that enable the capture, conveyance, storage, distribution, and efficient application of water to crops. Technological innovation is a key aspect, as technology levels vary depending on the type and size of the irrigation system. In the studied cases, sprinkler and drip irrigation systems predominate, characterized by their degree of pressurization, automation, and water-saving methods. Efficient infrastructure not only reduces water waste but also optimizes its application, thereby ensuring the sustainability of the system.

**Table 1.** SDGs and CSA-IRA Principles Related to the Sustainability Indicators of Irrigation Systems in Ecuador. Source from (Garcés and Padilla, 2020).

| | **SDG** | | **CSA - IRA Principles** |
|---|---|---|---|
| 2 | Zero Hunger | 1 | Contribute to food security and nutrition |
| 5<br>6 | Gender Equality<br>Clean Water and Sanitation | 2 | Contribute to sustainable and inclusive economic development and poverty eradication |
| 8 | Decent Work and Economic Growth | 3 | Promote gender equality and women's empowerment |
| 9<br>10 | Industry, Innovation and Infrastructure<br>Reduced Inequalities | 6 | Preserve and sustainably manage natural resources, enhance resilience, and reduce disaster risk |
| 11 | Sustainable Cities and Communities | 7 | Respect cultural heritage, traditional knowledge, and support diversity and innovation |
| 12 | Responsible Consumption and Production | 8 | Promote safe and healthy agricultural and food systems |
| 15<br>16 | Life on Land<br>Peace, Justice and Strong Institutions | 9 | Incorporate inclusive and transparent governance structures, processes, and grievance mechanisms |

### 3.2 Diagnostic Criteria and Critical Points of the Irrigation System

A total of 13 diagnostic criteria were identified, covering the seven sustainability attributes. Subsequently, 21 critical points were linked to the sustainability dimensions and system components (Table 2).

The critical points identified were organized according to the key sustainability attributes defined in the methodology. This classification enables a systematic analysis of the main weaknesses and opportunities for improvement in community-managed irrigation systems. The following section presents the findings corresponding to each sustainability attribute.

**Table 2.** Critical Points Identified for the Sustainability of Community Irrigation Systems in the Ecuadorian Andes.

| Attributes | Diagnostic Criteria | Critical Points | Sustainability Dimensions | System Elements |
|---|---|---|---|---|
| **1. Productivity** | System efficiency | Water loss across infrastructure components and at plot-level application | Environmental | Nature |
| | | | Technological | Infrastructure |
| | Water use performance | Use of irrigation in low-profit crops, generating limited employment opportunities | Economic | Community |
| | | | Social | |
| | | Lack of understanding of the relationship between investment benefits and economic return from production | Economic | Community |
| | | High investment cost per irrigation project | Economic | Community |
| **2. Stability** | Conservation, quality, and protection of resources | Reduced water availability due to degradation of water sources from anthropogenic activities | Environmental | Nature |
| | | | Technological | Infrastructure |
| | | Water body pollution due to agricultural activity | Environmental | Nature |
| | | Predominance of monoculture systems | Environmental | Nature |
| **3. Reliability** | Relationship between system revenues and costs | Insufficient self-managed resources for maintenance, repair, or replacement activities | Economic | Infrastructure |
| | | | Social | Community |
| **4. Resilience** | Risk prevention mechanisms | Lack of practices to prevent evapotranspiration | Environmental | Nature |
| | | No measures implemented to promote soil water retention | Environmental | Nature |

| | | | | |
|---|---|---|---|---|
| **5. Adaptability** | Irrigation technification | 40% of systems do not pressurize water for irrigation | Technological | Infrastructure |
| | Learning and training processes | Irrigation users lack knowledge of efficient water use alternatives | Technological | Community |
| | Capacity for change and innovation | Users reject technical and social changes | Technological | Community |
| **6. Equity** | Equitable distribution of water and gender | Water distribution does not consider crop type or surface area | Political | Community |
| | | Youth and women are not involved in organizational leadership | Political | Community |
| | Cost and benefit distribution | Tariffs are set per user without considering profitability or water consumption | Economic | Community |
| **7. Self-reliance (Self-management)** | Organizational capacity for system management and operation | Conflicts exist between irrigation systems and users over access and water use | Social | Community |
| | | Weak water governance between user organizations and the State generates conflicts | Political | Community |
| | | Leadership is often held by individuals unfamiliar with the dynamics of irrigation systems | Social | Community |
| | Participation in system management and operation | User participation exists, but contributions are considered per individual, not by surface area | Social | Community |
| | Dependency on inputs and external factors | Inefficient management of financial resources; lack of reinvestment in the system | Economic | Community |

## 3.3 Productivity

Irrigation systems ensure the timely and high-quality provision of water to increase productivity in irrigated areas Morris (2019); Contero and Cachipuendo (2021). Their evaluation requires consideration of technical, economic, and social efficiency, identifying critical points such as water waste due to infrastructure deterioration or poor management-issues that can be addressed through improvement actions and farmer training. Another key diagnostic criterion is water use performance, which includes critical aspects such as low crop profitability, cost–benefit ratios, and employment generation.

## 3.4 Stability

This attribute assesses the conservation, quality, and protection of resources. From an environmental perspective, critical points include water pollution, scarcity, and the predominance of monocultures. A sustainable community irrigation system requires sufficient availability of water in both quantity and quality, ensured by the protection of water sources and the promotion of biodiverse production systems. Soil sustainability is also a relevant factor within this attribute.

### 3.5 Reliability

Reliability is examined through a single diagnostic criterion: system income and opportunity costs. Critical points include operation and maintenance costs, and system-generated revenues (economic dimension). In the social dimension, insufficient or ineffective regulations for irrigation system management were identified.

### 3.6 Resilience

This attribute evaluates the system's ability to implement measures that reduce risks and strengthen resilience to climate change in agricultural contexts (Ward, 2022). In the environmental dimension, two critical points were identified: the lack of practices to reduce evapotranspiration and the absence of actions that enhance soil water retention. In the technological dimension, a critical point was the low level of irrigation technification.

### 3.7 Adaptability

The agricultural sector faces increasing competition for water due to being the largest consumer and the effects of climate change. This attribute assesses adaptive capacity through two criteria: strengthening learning and training processes for efficient water use, and the ability to innovate and adopt new irrigation technologies (van Opstal et al., 2022).

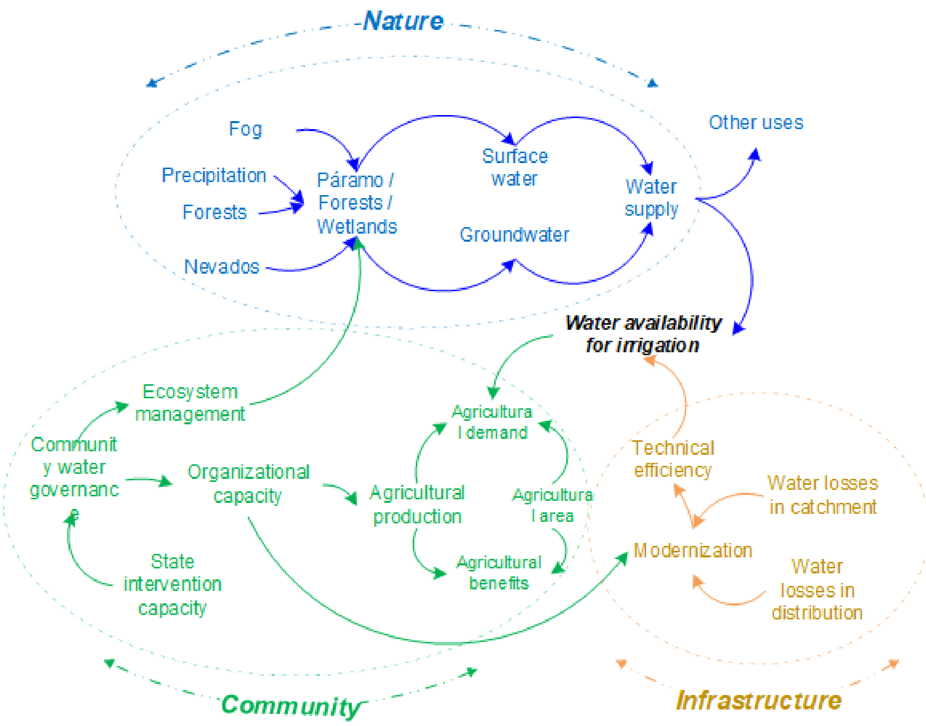


**Figure 2.** Model of operation of the community irrigation system.

### 3.8 Equity

Equity in water resource distribution is crucial to prevent conflicts and may be approached through the principle of "equitable distribution" (Elmusa, 1994). This attribute considers water allocation according to crop water requirements, irrigated area, and the participation of women and youth (social dimension). It also assesses tariff structures based on budget, irrigated surface, and crop profitability (economic dimension).

## 3.9 Self-sufficiency and Self-management

Community-managed irrigation systems face significant limitations regarding self-sufficiency and self-governance (Cortez, 2000). This attribute is analyzed through criteria such as the empowerment of irrigators to organize and manage financial resources (social–governance dimension), the level of user participation in system management (social dimension), and dependence on external inputs and factors (economic dimension).

## 3.10 Relationship Between Irrigation System Evaluation Indicators and the SDGs and CSA-IRA Principles

In addition to the internal analysis of irrigation systems, it is essential to link the defined indicators with global reference frameworks that guide sustainability. This integration allows for the evaluation of not only local performance but also the contribution of these systems to the fulfillment of international commitments (Table 3).

The following outlines the indicators corresponding to each MESMIS attribute:

**Productivity:** Seven indicators related to efficient water use and economic productivity were identified, covering social, environmental, economic, and technological dimensions. These indicators are linked to SDGs 8, 9, and 12, and CSA-IRA Principles 1, 2, and 6.

**Stability:** Four indicators reflect the importance of water availability and quality, associated with SDGs 2, 6, 11, and 15, and CSA-IRA Principles 1, 6, and 7.

**Reliability:** Two indicators cover aspects of the economic and social dimensions, related to SDGs 8, 10, and 16, and CSA-IRA Principles 2 and 9.

**Resilience:** Three indicators measure the system's capacity to withstand climate-related changes, linked to SDGs 3 and 5, and CSA-IRA Principles 1, 6, and 8.

**Adaptability:** Two indicators assess technological innovation and the adaptive capacity of irrigators, associated with SDGs 12 and 11, and CSA-IRA Principles 3 and 7.

**Equity:** Five indicators address the participation of women and youth, generational transition, and equitable water access, related to SDGs 2, 5, 9, 10, and 16, and CSA-IRA Principles 1, 3, and 7.

**Self-sufficiency and Self-management:** This attribute includes nine indicators related to governance and economic sustainability, aligned with SDGs 10 and 16, and CSA-IRA Principles 2, 7, and 9.

These indicators provide a comprehensive foundation for evaluating the sustainability of irrigation systems based on their contributions to the SDGs and CSA-IRA Principles, enabling the design of strategies aimed at continuous improvement.

A detailed description of the 31 defined indicators is presented below. This section includes the conceptual framework, calculation methods, and units of measurement, enabling their practical application in the sustainability assessment of community-managed irrigation systems.

#### System efficiency from water intake to plot

Calculated as the sum of the flows reaching irrigated plots divided by the intake flow at the water catchment point, expressed as a percentage of equation 1.

$$E(\%) = \frac{\sum Q_{\text{plots}}}{Q_{\text{intake}}} \times 100\% \quad (1)$$

#### On-plot water application efficiency

The ratio between crop water requirements and the amount of water applied through sprinklers or drip irrigation within the agricultural production unit (UPA), expressed as a percentage in equation 2. Where: EA = application efficiency; CWR = crop water requirement; $W_{\text{applied}}$ = water applied by the emitter (Playan, 1994).

$$EA(\%) = \frac{CWR}{W_{\text{applied}}} \quad (2)$$

#### Economic return per volume of water used

The monetary benefit generated per volume of irrigation water used (USD/$m^{-3}$) according with Ríos et al. (2016).

$$Y_1 = \frac{\text{Profit}(USD)}{V(m^3)} \quad (3)$$

**Water volume per employment generated**

Measures the number of agricultural jobs created per cubic hectometer (1 million $m^3$) of water used according to Hussain et al. (2007).

$$Y_2 = \frac{\text{Jobs}(units)}{V(m^3)} \quad (4)$$

**Benefit-cost ratio**

Calculated by dividing the total income from irrigated agricultural production over 10 years by the total costs of irrigation infrastructure and crop establishment according with equation 5.

$$\frac{B}{C}(USD) = \frac{\text{Total income}}{\text{Total cost}} \quad (5)$$

**Investment per hectare**

Expressed in USD/ha, this indicator enables standardized comparison of investment levels across irrigated areas.

**Investment per irrigation user**

Determined by dividing the total system investment by the number of irrigation users in equation 6. Being: MIUR = amount in dollars of investment per irrigation user; NUSR= number of users of the irrigation system.

$$MIUR = \frac{\text{Total Investment}}{NUSR} \quad (6)$$

**Water scarcity index**

Ratio between agricultural water demand and available supply at the catchment point, expressed as a percentage in equation 7. Where: D = demand ($m^3$), Ws = water supply ($m^3$). Ic = Scarcity index expressed in % (Ríos et al., 2016).

$$Ic = \frac{D}{Ws} \times 100 \quad (7)$$

**Water source conservation practices**

Quantified by practices such as reforestation, fencing, controlled grazing, and preservation of source areas, evaluated on a scale based on the number of practices implemented.

**Water Quality Index (WQI)**

Based on the Canadian Council of Ministers of the Environment WQI (CCMEWQI); its calculation was get by applying equation 8. F1 = scope (non-compliant variables), F2 = frequency (non-compliant tests), F3 = amplitude (degree of deviation).

$$CCMEWQI = 100 - \frac{\sqrt{F1^1 + F2^2 + F3^2}}{1{,}732} \quad (8)$$

**Crop diversity in UPAs**

The Shannon-Wiener Index was adapted for this calculation, as detailed below the excesses of each data out of range when compared to its threshold (Chidiac et al., 2023). Where $P_i$ is the proportion of individuals of the i-th crop and is calculated as follows $P_i = n_i/N$ , $N$ is the total number of individuals; $n_i$ is the number of plants per crop, $N$ the number of all plants of all crops and $S$ is the number of species (Valdez et al., 2018).

$$DC = -\sum_{i=1}^{S} P_i ln P_i \quad (9)$$

**O & M cost-income ratio**

To calculate this ratio, first add up the total annual operating, maintenance, and administrative costs and divide them by the net income from annual production, as detailed in equation 10.

$$\frac{C}{I} = \frac{\text{Operating,maintenance and administration costs}}{\text{Annual production}} \quad (10)$$

**Existence of regulations for the management and administration of the system**

This indicator evaluates the presence and application of internal regulations governing the operation, maintenance and administration of the irrigation system. These regulations are essential for effective governance, as they establish clear rules that guide decision making, promote participation and prevent conflicts (Perugachi and Cachipuendo, 2000). The absence or weakness of these rules indicates a low level of governance and may compromise the institutional sustainability of the system. It is expressed on a scale of 1 to 5 where: 1 = nonexistent, 2 = very weak, 3 = partially implemented, 4 = implemented with limitations, 5 = fully implemented and functional.

**Windbreak-based microclimate generation**

Ratio of farms implementing windbreaks to the total number of farms, expressed as a percentage.

**Organic matter incorporation**

Percentage of UPAs that incorporate organic matter into soils; may include complementary measures such as organic matter content and moisture level (Tácuna et al., 2015).

**Irrigation system technification level**

Based on infrastructure type, condition, and irrigation method, rated on a scale from 1 (low) to 5 (high).

**Knowledge of irrigation water needs**

Assessed through a qualitative scale: very good, good, fair, poor (Hussain et al., 2007).

**Acceptance of technological and social change**

Based on adoption of innovative practices in the past five years; desirable threshold is 7 or more (Fonseca-Carreño et al., 2016).

**Water distribution according to crop water requirements and area**

For this distribution (flow), the water requirements according to the type of crop ($q$) multiplied by the production area ($A$) are considered according to equation 11.

$$Q = q(L/s/ha) \times A(ha) \tag{11}$$

**Women and men participating in the organization's board of directors**

The participation of stakeholders in community systems is an essential condition, especially of women, as it ensures a more equitable management while guaranteeing their right to water. It is estimated as the percentage of women on the organization's board in relation to the total number of members of the organization (Chidiac et al., 2023).

**Young people participating in the organization's board of directors**

Similarly, the participation of young people in the leadership of the organization reflects social sustainability in terms of transition among the actors to assume water management. It is estimated as the percentage of young people on the organization's board with respect to the total number of members of the organization (Chidiac et al., 2023).

**Tariff regime based on the annual budget**

This indicator shows the economic sustainability and, depending on the components considered in the budget, the irrigation system can be efficiently managed and operated; it is expressed in terms of existence and degree of compliance on a scale of 1 to 5.

**Tariffs based on surface area and crop profitability**

This indicator evaluates whether the irrigation system applies differentiated tariffs according to the area cultivated and the profitability of the irrigated crops. A tariff structure based on these criteria promotes equity and economic efficiency in the management of water resources by considering the productive capacity of each agricultural unit. Unit of measurement qualitative scale from 1 to 5.

**Project management**

The organization's capacity to generate and finance projects is an indicator of the level of strategic and operational planning to improve the system. It is estimated as high: 5-4 projects; medium 3-1 projects and low 0 (Arnés et al., 2013).

**Coordination of actions with public institutions**

This indicator has to do with the governance of the systems and evidences in level of coordination of the organization with public institutions in order to achieve funding for projects, technical assistance, credit, training and other actions as part of the implementation of national or local policies and legal frameworks (Cobo et al., 2018). High level is estimated as: 5-4 actions; medium 3-1 actions and low 0 or none.

**Coordination of actions with civil society institutions**

Like articulation with public institutions, this indicator accounts for governance between civil society actors that may be neighboring irrigation organizations and from the same basin or sub-basin (Cobo et al., 2018). It is estimated as high level: 5-4 actions; medium 3-1 actions and low 0 or none.

**Level of democratic alternation of leadership**

This indicator will be estimated to the extent that it has been identified in the critical points of the organization. It is qualitative in nature. It is estimated by means of a categorical scale: high when what is established in the regulations regarding the election and renewal of leadership and decision-making is met; medium if it is partially met; and low, when it is not met (González et al., 2006).

**Leaders' level of knowledge of system management and administration**

This indicator measures the degree of knowledge that irrigation system leaders have about technical, social, environmental and governance aspects related to water use and management. An adequate level of knowledge is essential to ensure effective management, strengthen social sustainability and facilitate the implementation of regulations that prevent conflicts within and outside the organization (González et al., 2006). Unit of measurement: qualitative scale from 1 to 5, where: 1 = no knowledge, 2 = basic knowledge, 3 = medium knowledge, 4 = high knowledge, 5 = comprehensive and effectively applied knowledge.

**Level of equity in maintenance work contributions according to surface area**

The management and administration of the system requires the participation of all users; however, a critical point is the inequity in maintenance work. Participation in the work according to the surface area of each user is an indicator of equity. It is a qualitative indicator: high=yes, low=no.

**Irrigation system management**

This indicator reflects the practices that are necessary for efficient economic management of the system. Sustainability will be reflected by the existence of three basic instruments: planning, budget and accounting. Thus, it will be considered high when there are 3 instruments, medium when there are 2 and low when there is 1 or none.

**Reinvestment in the irrigation system**

For this indicator, the Reinvestment Return (RR) will be considered according to equation 12.

$$RR = \frac{\text{Total cash flows generated}}{\text{Total cash flows reinvested}} - 1 \qquad (12)$$

# 4 Discussion

This study successfully defined 31 indicators to assess the sustainability of community-managed irrigation systems in Ecuador, aligned with the Sustainable Development Goals (SDGs) and the CSA-IRA Principles. The definition of these indicators was achieved through a participatory methodology based on the MESMIS framework and the Delphi Panel technique, allowing the inclusion of the characteristics of irrigation systems within the Ecuadorian Andean context. In accordance with the proposed model, three essential components were identified: nature, community, and infrastructure. These closely interrelated elements reflect the ecological, social, and technical dynamics that shape irrigation management in Andean territories (Cachipuendo Ulcuango, 2021; Mazabel and Caldera, 2018). The model's characterization helped to understand how endogenous factors (organizational capacity, internal management, level of technification) and exogenous factors (climate variability, pressure on water sources) influence the sustainability of these systems.

The analysis of 13 diagnostic criteria and 21 critical points were distributed across seven sustainability attributes—productivity, stability, reliability, resilience, adaptability, equity, and self-management—revealed common structural weaknesses. For instance, under the productivity attribute, deficiencies were observed in system efficiency and the low profitability of irrigated crops (Morris, 2019; Contero and Cachipuendo, 2021), which affects both economic viability and the rational use of water resources.

System stability was found to be at risk due to the degradation of water sources and biodiversity loss, consistent with previous studies warning of the deterioration of paramo ecosystems (Llambí et al., 2012). Likewise, the limited implementation of conservation practices highlights the need for approaches that integrate environmental management with productive planning (Chile and Ortiz, 2021).

Regarding reliability, limited financial capacity for operation and maintenance, along with weak implementation of internal regulations, undermines the systems' economic and institutional sustainability (Perugachi and Cachipuendo, 2000). This finding aligns with research that links water governance to the existence of clear rules and effective participation mechanisms (Cobo et al., 2018).

**Table 3.** Sustainability Indicators in Relation to the SDGs and CSA-IRA Principles.

| Attributes | N° | Sustainability Indicators | | | | SDG - CSA-IRA Relationship | | |
|---|---|---|---|---|---|---|---|---|
| | | Description | Unit of Measurement | Minimum Value | Maximum Value | SDG | Goals | CSA-IRA Principles |
| **A1** | 1 | Efficiency of the system from the catchment to the field | % | 50 | 90 | 9 | 9.5 Increase scientific research and technological capacity. | 6 |
| | 2 | Efficiency of water application in the field | % | 50 | 96 | 9 | 9.5 Increase scientific research and technological capacity | 1 |
| | | | | | | 12 | 12.2 Achieve efficient use of natural resources | 6 |
| | 3 | Ratio of economic return to volume of water used | m3/$ | 0.1 | 10 | 8 | 8.4 Improve efficient and respectful consumption and production | 2 |
| | 4 | Ratio of volume of water used to the number of jobs generated | m3/day | 0.1 | 10 | 8 | 8.5 Achieve full employment and decent work. | 2 |
| | 5 | Benefit-cost ratio | unit | 0.1 | 1 | 8 | 8.2 Raise productivity through diversification, technology and innovation. | 1 |
| | 6 | Amount of investment per hectare | $/ha | 500 | 4000 | | | 2 |
| | 7 | Amount of investment per irrigation user | $/user | 100 | 4000 | | | |
| **A2** | 8 | Scarcity index | % | 40 | 90 | 9 | 9.5 Increase scientific research and technological capacity. | 6 |
| | | | | | | 11 | 11.1 Support for urban, peri-urban and rural linkages. | |
| | 9 | Source water conservation practices | unit | 1 | 5 | 11 | 11.4 Protect cultural and natural heritage. | 6 |
| | | | | | | 15 | 15.1 Ensure the conservation and sustainable use of ecosystems. | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10 | Water quality index | % | 50 | 100 | 6 | 6.3 Improve water quality. Reduce pollution and wastewater. | 6 |
| | 11 | Crop diversity in the UPAs | % | 50 | 100 | 2 | 2.5 Preserve the genetic diversity of seeds. | 17 |
| | | | | | | 15 | 15.4 Ensure the conservation of mountain ecosystems. | |
| **A3** | 12 | Ratio of operating, maintenance and administrative costs to revenues | unit | 1 | 5 | 8 | 8.2 Raise productivity through diversification, technology and innovation. | 2 |
| | | | | | | 10 | 10.1 Income growth of 40% of the poor population. | |
| | 13 | Existence of regulations for the management and handling of the system | unit | 1 | 5 | 16 | 16.b Strengthen the participation of developing countries in OOII. | 9 |
| **A4** | 14 | Generation of microclimates through windbreaks | % | 50 | 100 | 2 | 2.4 Promote sustainable and resilient agricultural practices. | 6 |
| | 15 | Incorporation of organic matter in the soil | % | 50 | 100 | | | 18 |
| | 16 | Technification level of irrigation systems | unit | 1 | 5 | 9 | 9.5 Increase in scientific research, technological capacity. | 18 |
| **A5** | 17 | Level of knowledge of the amount of water to be applied on the plot | unit | 1 | 5 | 12 | 12.2 Achieve efficient use of natural resources. | 3 |
| | 18 | Level of acceptance of technological and social changes | unit | 1 | 5 | 11 | 11.a Support linkage of urban and rural areas. | 7 |
| **A6** | 19 | Water distribution according to crop water requirements and surface area | unit | 1 | 5 | 2 | 2.4 Sustainable and resilient agricultural practices. | 1 |
| | 20 | Women and men involved in the organization's board of directors | unit | 1 | 5 | 5 | 5.5 Ensure access to sexual and reproductive health and rights. | 3 |
| | 21 | Young people who participate in the organization's board of directors | unit | 1 | 5 | 10 | 10.3 Ensure equality of opportunity | 3 |
| | | | | | | 16 | 16.b Promote and enforce laws and policies (human rights). | |
| | 22 | Tariff regime based on the annual budget | unit | 1 | 5 | 9 | 9.5 Increase scientific research and technological capacity. | 7 |
| | 23 | Pricing based on area and profitability of the crop | unit | 1 | 5 | | | |
| **A7** | 24 | Organizational capacity in project management | unit | 1 | 5 | 10 | 10.3 Ensure equality of opportunity | 7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | 16 | 16.b Promote and enforce laws and policies (human rights). | |
| 25 | Articulation of actions with public institutions | unit | 1 | 5 | 16 | 16.6 Create effective and transparent institutions. | 9 |
| 26 | Articulation of actions with civil society institutions | unit | 1 | 5 | | 16.7 Encourage citizen participation. | |
| 27 | Level of leadership alternation | unit | 1 | 5 | | | |
| 28 | Level of knowledge of management and handling of the system by leaders | unit | 1 | 5 | | | |
| 29 | Level of equity in labor input for maintenance based on surface area | unit | 1 | 5 | | | |
| 30 | Financial management of the irrigation system | unit | 1 | 5 | 16 | 16.5 Reduce corruption and bribery. | 2 |
| 31 | Reinvestment in the irrigation system | unit | 1 | 5 | | 16.6 Create effective and transparent institutions. | |

The resilience attribute revealed the absence of adequate mechanisms to cope with climate stress, such as irrigation technification and soil moisture conservation practices (Ward, 2022), which compromises the adaptive capacity of these systems in the face of extreme events. In this regard, technological innovation emerges as an urgent necessity.

In terms of adaptability, limited appropriation of knowledge and technologies by irrigators was evident, restricting their ability to respond to socio-environmental changes (van Opstal et al., 2022). This limitation also affects the equity attribute, particularly due to the low participation of youth and women in decision-making spaces, which hampers generational renewal and social inclusion (Elmusa, 1994; Chidiac et al., 2023).

Finally, in the dimension of self-management, the analysis revealed weak institutional articulation and deficiencies in leadership and financial management, reflecting low organizational self-sufficiency. This situation compromises community governance and threatens long-term sustainability (Cortez, 2000; González et al., 2006).

The alignment of the indicators with 10 SDGs and 7 CSA-IRA Principles (Garcés and Padilla, 2020; FAO, 2014) reinforces the strategic contribution of these irrigation systems to sustainable development. Thus, the methodological proposal offers an operational tool for evaluating and intervening in community-managed irrigation systems from a holistic, dynamic, and context-sensitive perspective (Pérez-Serrano et al., 2021).

Nevertheless, certain limitations must be acknowledged: the validation of the indicators was restricted to the Ecuadorian Andean context; the application of the Delphi Panel technique entails subjective biases; and the linkage to the SDGs and CSA-IRA is conceptual in nature. These limitations highlight the need for future research to further the empirical validation and adapt the model to other geographical contexts.

# 5 Conclusions

This research developed a comprehensive methodological framework for assessing the sustainability of community-managed irrigation systems in the Ecuadorian Andes by defining 31 indicators organized into seven key attributes: productivity, stability, reliability, resilience, adaptability, equity, and self-management. These indicators were grouped into five analytical dimensions-environmental, social, economic, technological, and political-and structured around three core components of the system: nature, community, and infrastructure.

The findings reveal multiple critical issues affecting the sustainability of these systems, such as water use inefficiency, weak governance, low participation of key groups, and limited adaptive capacity to climate change. Through the participatory MESMIS approach and the Delphi Panel technique, the study achieved a contextualized characterization of the systems, enabling their alignment with ten SDGs and seven CSA-IRA Principles.

At the regional level, the results offer a practical tool for community stakeholders, public institutions, and policymakers to design, monitor, and evaluate water management strategies in irrigated territories. At the global level, this proposal contributes to international sustainability reporting, positioning community-managed irrigation systems as key actors in resilient and inclusive agriculture.

However, it is recommended that the indicators be empirically validated in other contexts and that broader stakeholder engagement be promoted during their implementation to strengthen applicability and impact. This study thus represents an important step forward in constructing integrative methodological frameworks for sustainable water management in agriculture and in reducing pressure on strategic ecosystems such as the paramo.

## Authors' contributions

C.C.: Conceptualization, data processing, discussion. M.I.: Conceptualization, data processing, discussion. N.R.: Conceptualization, data processing, in relation to the indicators with the SDGs and IRA principles.

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Agricultural Sciences

# Scrutinizing the efficacy of plant extracts and bactericidal agents applied against *Xanthomonas axonopodis*, the causative agent of bean blight

# Estudio de la eficacia de los extractos de plantas y agentes bactericidas aplicados contra *Xanthomonas axonopodis*, el agente causante del tizón del frijol

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**Abstract**

Common bean (a major staple seed crop and legume) is susceptible to bean blight (*Xanthomonas axonopodis*). The study controlled bean blight in the laboratory and screen-house using botanicals and bactericides. Completely randomized and replicated design was used and recorded percentage germination, number of leaves, shoot length, seed weight, shoot fresh weight, disease incidence, and severity. *In vitro*, control of the pathogen depended on the application of antibiotics: tetracycline, cephalosporin, lincomycin, and erythromycin in order of efficacy, giving 52.2–100 % inhibition of the pathogen. *In vitro*, aqueous extracts of *Eucalyptus globulus*, *Aframomum melegueta*, *Ricinus communis*, and *Acmella oleracea* effectively inhibited 25.0–62.5 % of the bacterial growth. In screen-house, effects of chemical bactericides on *Xanthomonas* species revealed a significant difference in the number of leaves at 49 days after inoculation (DAI). Also, percentage inhibition of *Xanthomonas* species by the bactericides ranged from 46.2-97.5 % from 6-56 DAI. Shoot lengths were significantly different under the influence of plant extracts at 35 DAI and 49 DAI. Plant extracts caused 36.4-90.9 % percentage inhibition of the pathogen from 6-56 DAI. Formulation of agricultural applications using these control agents is required.

***Keywords***: Bactericides, common bean blight, plant protein, *Xanthomonas campestris*, *Xanthomonas phaseoli*.

**Resumen**

El frijol común (un cultivo básico de semilla y una leguminosa importante) es susceptible al tizón del frijol (*Xanthomonas axonopodis*). El estudio controló el tizón del frijol en el laboratorio y en el invernadero utilizando productos botánicos y bactericidas. Se utilizó un diseño completamente aleatorizado y replicado y se registró el porcentaje de germinación, el número de hojas, la longitud del brote, el peso de la semilla, el peso fresco del brote, la incidencia de la enfermedad y la severidad. Por medio del *in vitro*, el control del patógeno dependió de la aplicación de antibióticos: tetraciclina, cefalosporina, lincomicina y eritromicina en orden de eficacia, dando un 52,2–100 % de inhibición del patógeno. Los extractos acuosos *in vitro* de *Eucalyptus globulus*, *Aframomum melegueta*, *Ricinus communis* y *Acmella oleracea* inhibieron eficazmente el 25,0–62,5 % del crecimiento bacteriano. En el invernadero, los efectos de los bactericidas químicos en las especies de *Xanthomonas* revelaron una diferencia significativa en el número de hojas a los 49 días después de la inoculación (DDI). También la inhibición porcentual de las especies de *Xanthomonas* por los bactericidas osciló entre el 46,2 % y el 97,5 % entre los 6 y los 56 DDI. La longitud de los brotes fue significativamente diferente bajo la influencia de los extractos vegetales a los 35 y 49 DDI. Los extractos vegetales causaron una inhibición porcentual del patógeno del 36,4 % al 90,9 % entre los 6 y los 56 DDI. Se requiere la formulación de aplicaciones agrícolas utilizando estos agentes de control.

***Palabras clave***: Bactericidas, tizón común del frijol, proteína vegetal, *Xanthomonas campestris*, *Xanthomonas phaseoli*.

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# 1 Introduction

Common bean (*Phaseolus vulgaris* L. in the plant family Fabaceae) is a principal grain legume globally. Its varieties include French bean, haricot bean, salad bean, snap bean, string bean, and kidney bean. Around 2019-2022, approximately 28 million metric tonnes of dry common beans were produced globally (Kadege et al., 2022; FAOSTAT, 2024). FAO (1999) and Porch et al. (2013) acknowledged that the market value of these beans exceeds that of all the other legumes combined. While its yield data is unreliable in Africa, the continent accounted for 7.8 million hectares (circa 25 %) of the total global area under common beans.

Beans are a staple food in millions of households in Africa. They are a major source of income and food security in sub-Saharan Africa, especially in eastern Africa (Ethiopia, Kenya, Burundi, Tanzania, Uganda), and West Africa - especially in Nigeria (Howard et al., 2005; CABI, 2022; FAO, 1999; Kadege et al., 2022). Beans are cultivated worldwide for their edible seeds/pods and occasionally for their edible leaves and straw fodder.

Beans are rich in dietary fibre, protein, vitamins (like vitamin A and vitamin C), and minerals (i.e., iron, zinc, copper, potassium, calcium, and magnesium). Common beans account for 8–10 % protein per 100 g daily intake and they are rich in all the essential amino acids, especially lysine and tryptophan, but deficient in methionine. Beans have very low fat and unhealthy cholesterol content (CABI, 2022; Câmara et al., 2013; Chen et al., 2021).

Câmara et al. (2013) and Kadege et al. (2022) pointed out that this bean is a functional food because it helps improve our health. This is tied to its high levels of phenols, starch, vitamins, and fructo-oligosaccharides that combat distresses like heart disease, sugar-related diseases, and various oncological conditions. Beans could be preserved by drying, cooking, canning, or processed as gluten-free wheat flour.

The production constraints related to beans include diseases, insect pests, low soil fertility (mainly phosphorus deficiency), abiotic stresses (especially drought), inadequate adaptation of introduced varieties, low access to inputs, and inappropriate management of production processes (Kimani et al., 2005; Akibode and Maredia, 2011; Porch et al., 2013; Beebe et al., 2014; OECD, 2016; Mondo et al., 2019; Kadege et al., 2022).

Bean blight or common bean blight (caused by *Xanthomonas axonopodis* pv. *phaseoli* in the bacteria family Lysobacteraceae (i.e. Xanthomonadaceae)) is one of the five major bacterial diseases of common beans. It attacks the foliage, pods, seeds, and stems (ISTA, 2007; Muedi and Fourie, 2014; Chen et al., 2021). Karavina et al. (2011) and Manju et al. (2024) agreed that bean blight is more severe at temperatures between 25-35 °C, especially when coupled with heavy rainfall, and high relative humidity. Both authors estimated that this infection can result in 40 % yield loss.

Due to this significant bean yield loss, researchers have been trying to develop sustainable management options for bean blight. Chen et al. (2021) pointed out that insufficient options (either chemical or biological) exist against bacterial diseases. Thus, the options available to producers are limited to cultural practices. They confirmed that copper-based fungicides (cum bactericides), streptomycin, kasugamycin, and manganese-based foliar fertilizers are effective against plant-infecting bacteria.

Karavina et al. (2011) and Muedi and Fourie (2014) affirmed that copper-based bactericides (like copper oxychloride, copper oxide, copper sulphate, copper hydroxide), and potassium di-ethyl-dithio-carbamate can effectively control bacterial foliar infections. Some plant-based agents like essential oils from various plants may be effective against bean blight. Synthetic antibiotics (i.e. streptomycin and kasugamycin) successfully control external bacterial agents even though no seed treatment agent has been developed to completely eradicate *X. axonopodis* situated inside seeds.

Câmara et al. (2013) and Porch et al. (2013) stated that the common bean is under-researched especially in Africa. This study presents effective solutions to address the urgent problem of *X. axonopodis* infections in common beans. The aim is to assess the effectiveness of different plant extracts and bactericidal agents against this pathogen, and show their potential as viable management strategies.

# 2 Materials and Methods

## 2.1 Site of the study

This study was performed in Nigeria at the Alex Ekwueme Federal University Ndufu-Alike, Abakaliki (6.069°N by 8.199°E). Legumes like cowpea, common bean, pigeon pea, Bambara groundnut, soya bean, mung bean, sword bean, and so on are widely cultivated in all the agroecological zones of West Africa including Nigeria.

However, the yields of beans are mostly below the global average due to various production constraints, among which are pests and diseases. The ecological requirements for the production of beans are similar to those required for *Xanthomonas axonopodis* (Figure 1). This is a conundrum that leaves farmers and researchers feeling impotent against this pathogen.

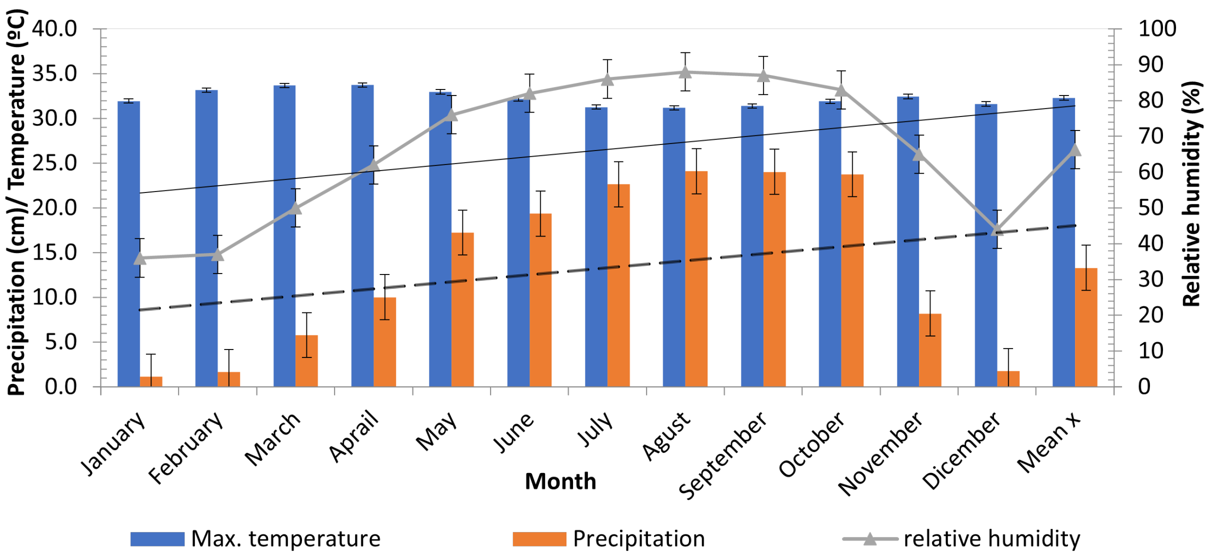


**Figure 1.** The typical temperature, relative humidity, and rainfall patterns for the study sites at Abakaliki, Ebonyi State. Figure modified from Ndifon (2022).

## 2.2 Preparation of the plant extracts

The plant organs (like *Ricinus communis* seed soap, flowers of *Acmella oleracea*, *E. globulus* resin, and *A. melegueta* seeds) utilized for the control of common bean bacterial blight were sourced from Abakaliki and its surroundings. They were washed with tap water, and surface sterilized with 1% sodium hypochlorite for 5 minutes. These plant materials were macerated into paste/powder using a mortar and pestle. Each plant material contained 165 g plant tissue per liter of sterile distilled water and it was extracted for 24 hours. For clarity, the plants used included castor bean which is *Ricinus communis*, toothache plant which is *Acmella oleracea*, blue gum which is *Eucalyptus globulus*, and alligator pepper which is *Aframomum melegueta*.

## 2.3 Isolation of the bacteria pathogen

The seeds and shoots of kidney beans utilized for the trial were initially obtained from Jos (in Plateau State), Nigeria. The plant organs were surface sterilized using 1% sodium hypochlorite for two minutes and rinsed with sterile distilled water. Autoclaved nutrient agar enriched with glucose-containing fluconazole (1 g per L) was used for the isolation of the pathogen (Kado and Heskett, 1970). Three seeds per Petri dish were placed on the agar aseptically.

The plates were subsequently incubated at 28±2°C for 24 hours. The bacteria growths that were noticed were individually sub-cultured and observed for colony similarity. Luckily, only one type of bacteria colony was isolated from the bean tissues. Sub-culturing was continued to purify the isolate. The pathogen was stored at 4°C and used subsequently for morphological and biochemical characterization of the pathogen (Sinclair and Dhingra, 1995; Grimault et al., 2024).

## 2.4 Total microbial count

A serial dilution to $1 \times 10^6$ of the homogenate was made in sterile test tubes using the direct suspension method (Ordóñez et al., 2023). One mL of the serially diluted bean sample was pipetted into each serially marked Petri dish.

Nutrient agar was used for the total count. The streak plate method was utilized to culture the bacteria. Autoclaving was carried out at 120 °C, 15 psi for 15 minutes. At the end of incubation, the colonies were counted and CFU per mL of the suspension were calculated, giving a good estimate of the viable count of the bacterial CFU.

## 2.5 Characterization and identification of the bacteria isolates

Phenotypic and biochemical tests were relied on for the characterization and identification of the pathogen (ISTA, 2007; Rajyalakshmi et al., 2016; Ordóñez et al., 2023; Grimault et al., 2024). A combination of tests is preferable for in-depth identification. We utilized the phenotypic and biochemical test options for this pathogen.

### 2.5.1 Gram staining of the bacteria isolates

The bacteria colony was smeared on a clean glass slide and flamed briefly over a Benson burner. Aqueous crystal violet solution (0.5%) was added to the smeared section for 30 seconds and washed with water for one minute. It was replaced with Gram's iodine solution for one minute and rinsed using a wash bottle. Rapid de-colorization with 95% ethanol was carried out.

The smear was counterstained with safranin for 10 seconds, washed using a wash bottle, dried, and observed under the microscope for the presence of staining of the bacteria (Rajyalakshmi et al., 2016; Ordóñez et al., 2023).

### 2.5.2 Motility test for the bacteria isolates

The test was carried out using nutrient + glucose broth, in a semi-solid medium. For this motility test, this medium was prepared according to the manufacturer's instructions. The broth was poured into the test tubes and inoculated with the bacteria growth using the stab inoculation technique. The stab inoculation was done using a young growth (cultured for 24 hours). The incubation was carried out at 28±2°C. The tubes were examined for growth and signs of motility. If the bacteria are motile and they are inoculated using stab technique, the bacteria growth spreads laterally in the medium with passage of time.

### 2.5.3 Morphological characterization of the bacteria isolates

The bacteria were streaked on the respective medium and incubated at 28±2°C for 24 hours. At the end of the incubation, the colonies were observed for morphological and cultural characteristics including the form of the margin, nature of the surface, texture, elevation, shape, color, and transparency/translucency of the colony (Wogu and Ofuase, 2014; Rajyalakshmi et al., 2016).

### 2.5.4 Biochemical characterization of the bacteria isolates

The bacteria isolate was identified using the following biochemical tests: carbohydrate utilization (carried out using glucose for carbohydrate utilization test), catalase, urease, aesculin, oxygen usage, hydrogen sulphide, starch hydrolysis, nitrate reduction, oxidase, KOH, and urease tests (Saddler and Bradbury, 2005; Porch et al., 2013; Wogu and Ofuase, 2014; Rajyalakshmi et al., 2016; Grimault et al., 2024).

The biochemical and morphological characteristics of the isolate revealed that the pathogen is *X. axonopodis* (strongly tied to pathovar. *phaseoli* based on literature/manuals, pathogenicity, and contrasting with likely bacteria agents of bean) (Table 1). Pathogenicity tests were carried out *in vivo* using these isolates, and the kidney bean was highly susceptible (Grimault et al., 2024).

## 2.6 Preparation of the bacteria strain used for both *in vitro* and *in vivo* trials

Bean blight or common bean blight is caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin et al. (syn. *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye; or *Xanthomonas phaseoli* pv. *fuscans* (Burkholder) Starr & Burkholder).

The plates were incubated on nutrient agar at 28±2°C for 24 hours. Serial dilution to $1 \times 10^6$ of the homogenate culture was made in sterile test tubes. A 0.5 McFarland standard was used to create inoculum densities of $1.0 \times 10^6$ CFU $mL^{-1}$ using the direct suspension method in saline water (containing NaCl 8.5 g $L^{-1}$) (Wogu and Ofuase, 2014; Ordóñez et al., 2023; Grimault et al., 2024).

## 2.7 *In vitro* test

### 2.7.1 Evaluation of bactericides against *X. axonopodis*

Antibiotic resistance patterns and antimicrobial activity of bacterial isolates were studied *in vitro* using chemical bactericides. The antibiotics included erythromycin, tetracycline, cephalosporin, and lincomycin (all at 500 mg of the commercial preparations) per liter (Wogu and Ofuase, 2014; Rajyalakshmi et al., 2016; Ordóñez et al., 2023). A negative control was included using only sterile distilled water as placebo.

Nutrient agar enriched with dextrose was utilized for this test. The entire agar surface was covered with the antibiotic agent using the spread plate technique. The rates were 0%, 50%, or 100% concentrations. A cork-borer (diameter of 6 mm) was utilized to aseptically make a hole in the center of the agar. *X. axonopodis* suspension (50 $\mu$L) was applied to the well.

The antibiotic diffused into the agar and inhibited the growth of the pathogen. Incubation of the bacteria was carried out at 28±2°C for 24 hours. Observation of the plates for zones of inhibition was carried out; the diameter (mm) was measured and recorded using a pair of calipers (Saddler and Bradbury, 2005; Mounyr et al., 2016).

### 2.7.2 Evaluation of plant extracts against *X. axonopodis*

Antibiotic resistance patterns and antimicrobial activity of bacterial isolates were studied *in vitro* using plant extracts. The plant extracts included *E. globulus*, *A. oleracea*, *A. melegueta* and *R. communis*. A negative control was included among the treatments. A negative control is a treatment that is inoculated with the pathogen and is not controlled/inhibited at all. A negative control shows or suffers the full effects of the pathogen.

A nutrient agar medium with fluconazole was utilized for this test. The test was replicated three times. The entire agar surface was inoculated using a spread plate technique with 50 $\mu$L of *X. axonopodis* suspension prepared above. A cork-borer (diameter of 6 mm) was utilized to aseptically make a hole in the center of the agar and 100 $\mu$L of the plant extract was applied to the well.

The antibiotic diffused into the agar and inhibited the growth of the pathogen. Incubation of the plates was carried out at 28±2°C for 24 hours. Observation of the plates for zones of inhibition was carried out, and the diameter of growth (mm) was measured using a pair of calipers and recorded accordingly.

## 2.8 *In vivo* trials

**Trial 1: Effects of plant extracts on common bean blight infecting kidney bean: consequences on growth and yield of bean plants in the screen-house**

The plant extracts (prepared above) were utilized at the dose rates of 0.0, 50, and 100% prepared through arithmetic progression dilutions. The *in vitro* CRD trial was carried out using nine treatments, which included a control, *A. oleracea* (50% and 100%), *E. globulus* (50% and 100%), and *R. communis* (50% and 100%). The plants were cultivated for 90 days before the termination of the trial.

**Trial 2: Effects of bactericides on *X. axonopodis* infecting kidney bean: consequences on growth and yield of this blight on bean plants in the screen-house**

The CRD pot experiment (replicated three times) had the following treatments: a negative control, tetracycline (0, 50, and 100%), lincomycin (0, 50, and 100%), and cephalosporin (0, 50, and 100%). The plants were cultivated for 90 days before the termination of the trial. Each fungicide was prepared at the rate of 500 mg $L^{-1}$ from commercial formulations.

### 2.9 Data collection and analysis

Percentage germination was calculated by dividing the total number of seeds that germinated over the total number of seeds sown per plot times 100%. Seedling vigor was obtained using a scale (Ndifon, 2023). Disease severity was obtained using the scale presented in Table 1.

The percentage inhibition of the pathogen was calculated using the equation 1.

$$PI = \left(\frac{C-T}{C}\right) * 100\% \quad (1)$$

Where, $PI$ = Percentage of inhibition of the growth of the pathogen
$C$ = Radius covered by the pathogen in the negative control
$T$ = Radius covered by the pathogen in the treated plate

The data collected included: percentage germination, seedling vigor, number of leaves, shoot length, stem diameter, number of branches, seed weight, shoot fresh weight, number of pods, incidence, and severity of blighting. The incidence of blighting was recorded after counting the infected plants divided by the total number of plants in the accessed treatment times 100%. The data were subjected to an analysis of variance test and the means were separated using Duncan's multiple range test (DMRT) ($p \leq 0.05$).

**Table 1.** Disease severity scale.

| Rating | Severity % | Description of severity on shoot/leaves |
|---|---|---|
| 0 | 0-0.99% | No lesions, good color of leaves and vigorous plants |
| 1 | 1-3.0% | Hard to discern visible symptoms |
| 2 | 3.1-10% | A few blighted lesions on plant |
| 3 | 10.1-20% | Slightly more blighted lesions than in 1 |
| 4 | 20.1-30% | Small blighted lesions with limited sporulation |
| 5 | 30.1-40% | Plants having few large blighted surfaces and sporulation |
| 6 | 40.1-50% | Abundant and generally large sporulation blighted surfaces |
| 7 | 50.1-60% | Plants having large lesions, chlorotic, and necrotic tissue |
| 8 | 60.1-70% | Highly discernable sporulation and coalescing lesions |
| 9 | 70.1-80% | Large sporulation, coalescing lesions, and few fallen leaves |
| 10 | 80.1-90% | Leaf fall very rampant, large sporulation, and coalescing lesions |
| 11 | 90.1-100% | Highest leaf fall, drooping leaves, death plants |

## 3 Results and Discussion

The results of the phenotypic characterization and biochemical tests carried out on the bacteria are presented in Table 2. These tests together with literature (Schaad et al., 2001; Lacy and Lukezic, 2004; Saddler and Bradbury, 2005; ISTA, 2007; Wogu and Ofuase, 2014; Rajyalakshmi et al., 2016; Ordóñez et al., 2023; Grimault et al., 2024), were used to confirm the identity of the bacterium *Xanthomonas axonopodis*.

The attempt to control the bacterium *in vitro* using bactericides was successfully carried out and presented in Figure 2. The efficacy of tetracycline (at 50% and 100% concentrations) was significantly superior to other treatments (Table 3). The other treatments (erythromycin 50%, lincomycin, and cephalosporin (each at 50% and 100% concentrations) were superior to the control. The bactericides achieved excellent inhibition of the bacteria to the tone of 52.2-100% inhibition based on the amount of growth inhibition zone.

**Table 2.** Characterization of the bacterial strain used.

| Characterization for the bacterium specimen | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Morphological characteristics** | Gram staining | Motility | Margin of the colony | Surface | Texture | Elevation | Colony shape | Color | Transparency | | |
| **traits** | - | + | Entire | Shiny | Smooth | Raised | Circular, convex, irregular | Orange-yellow | - | | |
| **Biochemical characteristics** | Gluscose/ Carbohydrate utilization -glucose | Catalase | Hydrogen sulphide production ($H_2S$) | Starch hydrolysis | Oxidase | KOH test | Fermentation in Oxi-Fem | Hydrolysis of Aesculin | Aerobic / oxygen | Nitrate reduction | Urease |
| **traits*** | + | + | + | + | - | + | - | + | + | - | - |

*Traits could be positive (+) or negative (-). The + or – indicates the type of response to the agent used to affect the organism. Expected responses are tagged + and lack of expected responses are tagged -.

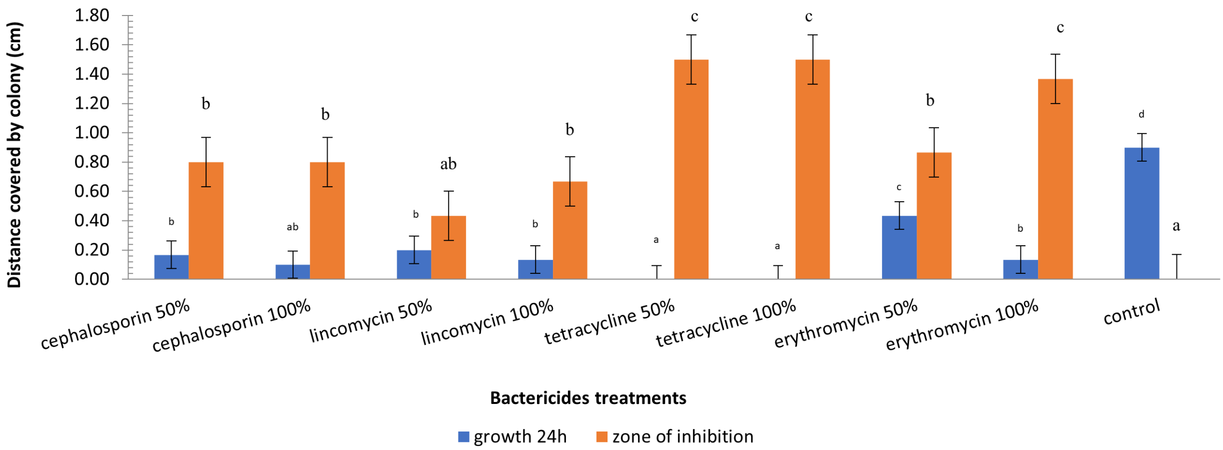


**Figure 2.** Effect of synthetic bactericides on *X. axonopodis in vitro*.
*Means overshadowed by the same letter(s) are statistically similar using DMRT ($p \leq 0{,}05$).

The botanicals effectively controlled *X. axonopodis in vitro*, leading to a reduction of the radial growth (Table 3). The plant extracts caused between 28.8-62.5 % inhibition of the pathogen. *E. globulus* 100 %, *A. melegueta* 100 % and *R. communis* 100 %, *R. communis* 50 %, then *A. oleracea* 100 % were the best treatments applied against *X. axonopodis in vitro*.

Meanwhile, controlling the bacterium *in vitro* using botanicals was equally successful and is presented in Figure 3. The zone of inhibition was recorded 48 hours after inception. It shows that *E. globulus* (at 100 % concentration) produced the largest zone of inhibition, followed by the *A. oleracea* (at 100 % concentration), then *A. melegueta* (at 100 % concentration), and *Eucalyptus* sp. 50 % well before *A. melegueta* (at 50 % concentration).

The effects of chemical bactericides on *Xanthomonas* species revealed a significant difference in the number of leaves between the treatments at 49 DAI (Figure 4). No significant differences existed between the shoot lengths. The percentage of inhibition of *Xanthomonas* species by the bactericides ranged from 46.2-97.5% over time. The inhibition was highest at the beginning of the trial (20 DAI) for all the chemicals, but this reduced with time.

Plant extracts caused no significant difference between the treatments based on the number of leaves produced in the screen house (Figure 5). The shoot lengths were significantly different under the influence of plant extracts at 35 DAI, and 49 DAI. Nevertheless, these differences were inconsistent with time. The percentage of inhibition of the pathogen varied from 36.4-90.9% with time. It can be mentioned that the 50% and 100% rates of *E. globulus* are the best treatments, followed by *R. communis* 100% rate, and *A. oleracea* 50%.

**Table 3.** Inhibition of the growth of *X. axonopodis* by selected plant and chemical bactericides *in vitro*.

| Chemical bactericides | % inhibition |
|---|---|
| Cefalosporina 50% | 81.1 |
| Cefalosporina 100% | 88.9 |
| Lincomicina 50% | 77.8 |
| Lincomicina 100% | 85.6 |
| Tetraciclina 50% | 100.0 |
| Tetraciclina 100% | 100.0 |
| Eritromicina 50% | 52.2 |
| Eritromicina 100% | 85.6 |
| **Plant extracts** | **% inhibition** |
| *Aframomum melegueta 100%* | 53.8 |
| *Aframomum melegueta 50%* | 28.8 |
| *Eucalyptus globulus 100%* | 62.5 |
| *Eucalyptus globulus 50%* | 33.8 |
| *Acmella oleracea 100%* | 46.3 |
| *Acmella oleracea 50%* | 33.6 |
| *Ricinus communis 100%* | 53.8 |
| *Ricinus communis 50%* | 50.1 |

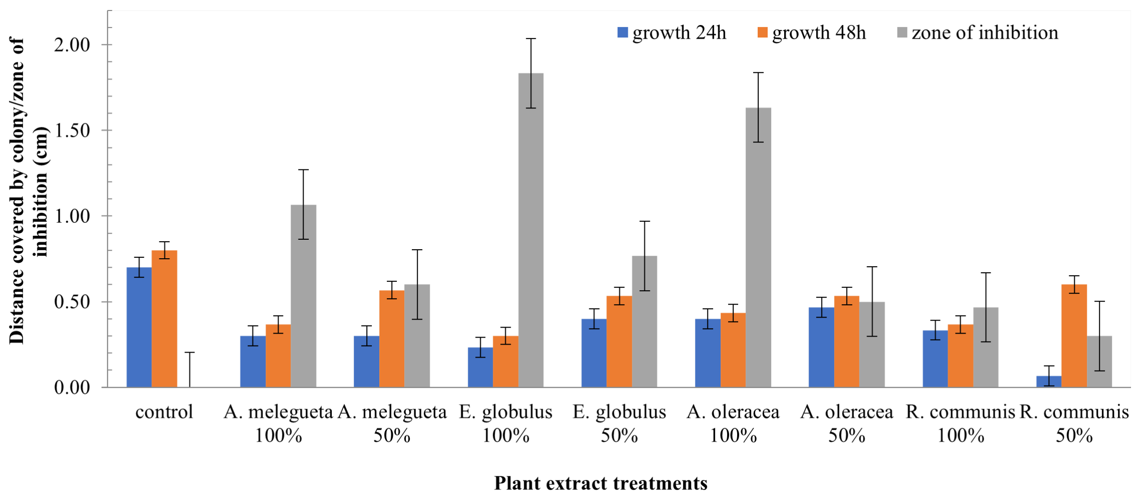


**Figure 3.** Effect of botanicals on *X. axonopodis in vitro*.

Fresh pod weight and seed weight were not significantly different between the treatments when chemical bactericides were applied against the bacterium (Figure 6). There were differences between treatment means when the fresh pod and seed weights were compared in a non-statistical manner. It is likely that for these beans with indeterminate growth patterns, the yields could be significantly different later. The fresh shoot weight varied significantly from the control.

Likewise in Figure 7, the fresh pod weight and seed weight were generally lower than those in the trial where chemical bactericides were applied. The time to first flower produced was longer for plant extract plots compared to the time for chemical plots. The reason for this was not clear. It is probable that the chemical agents boost time to flowering or pod production. Maybe some factor was present in plant tissue that favors continuation of vegetative growth. These are basically untested conjectures. There were visible differences between the seed, shoot, and pod weights which turned out not to be significant.

Finally, Figure 8 is attached to help readers appreciate the magnitude of the damage bacteria diseases can pose to plants including beans. It shows the dead seedlings planted in plot trials and damaged tissues of the beans during the experiment. The quality of *in vitro* pictures was low so they are not included. The trial will be conducted in the field when safer preparations of chemicals are available.

Bean blight is indeed a devourer of plants, which leaves the farmer without leaves or fruits. It devours the fodder, hay, and seeds seamlessly and mercilessly. Unfortunately, perfect control may be unattainable once the disease is in the field. This means no export or import to many countries where the producers could get premium prices. Researchers are up and doing in the struggle to ensure healthy production. This can enable producers to make a profit, value their crops more, and thus adopt modern production paraphernalia.

Howard et al. (2005) contended that bean blight agents could be controlled using chemical fungicides (cum bactericides). Buruchara et al. (2010) agreed that seed treatment using Copper compounds and Streptomycin, as well as restriction of field operation during rains has proven effective. Muedi and Fourie (2014) affirmed this opinion but reiterated that these chemicals cannot eliminate established bacteria from crops/fields. They believed that such chemicals could only reduce the spread of bacterial disease. These views were corroborated in this present study *in vitro* and *in vivo*.

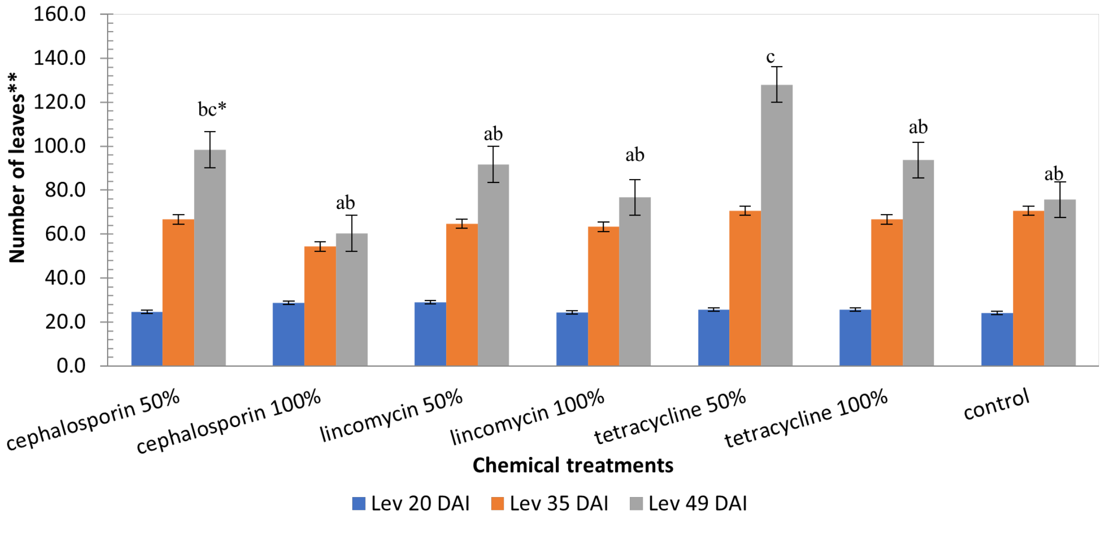


**Figure 4.** Effects of synthetic bactericides on crop growth and *X. axonopodis* in the screen-house. Note: ** Lev = leaves, Sht = shoot, %INH = percentage of inhibition. *Means overshadowed by the same letter(s) are statistically similar using DMRT ($p \leq 0{,}05$).

But Karavina et al. (2011) pointed out that the use of these measures by researchers is only possible in the short run. They stated that control of bean blight is possible by using disease-free seeds and chemicals. Thus, we embarked on this research and successfully proved the usefulness of bactericides in the production of common beans.

Research on control of bacterial blight of beans using plant extracts is rare. Control of fungi has been severally proven with the aid of plant materials. Waware et al. (2017) reported that aqueous extracts of marigold (*Tagetes erecta*) flowers exhibited potential antifungal activity against *Sclerotium rolfsii* under greenhouse conditions.

Sanasam et al. (2018) proved that plant extracts of garlic and turmeric inhibited (67.7%) *S. rolfsii*. Ndifon et al. (2022) revealed that aqueous extracts made from ginger and garlic for seed and soil dressing significantly controlled *Fusarium* wilt during the cultivation of *Solanum aethiopicum*.

Hussain et al. (2009) revealed that six plant extracts, including *Eucalyptus camaldulensis*, inhibited fungi species by suppressing the mycelia growth. Ndifon and Lum (2021) reported that all the plant extracts (including *Eucalyptus globulus*) assayed inhibited the growth of *Aspergillus niger* significantly compared to the control. The plant species applied in this study have all proven very effective in our laboratory against fungi. They showed much promise in this study but they could not surpass synthetic bactericides. Chemical bactericides still have a role in bean blight management.

Since bacterial diseases of plants are often treated using fungicides for instance copper oxides and copper oxychlorides, much research on use of plant extracts against fungi has been carried out. This study was carried out with the hope that some plant extracts that have been effective against fungi will also be effective against bacteria. This could be a plus for pathogen control if the control materials are dual-purpose pesticides.

Many researchers have raised alarm about the safety of chemical pesticides to man, animals, and the environment. Attempts to offer farmers different control agents are rare as far as bean blight is concerned. Cultural control and the use of resistant materials may be feasible for small plots or isolated fields. Breeding for resistance is made difficult because of the high rate of self-pollination in beans.

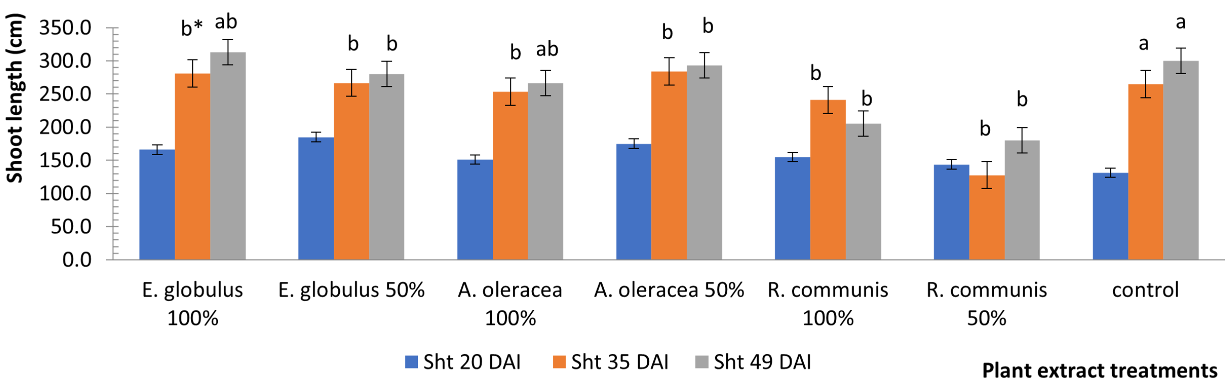


**Figure 5.** Effects of plant extracts on crop growth and *X. axonopodis* in the screen-house.
*Means overshadowed by the same letter(s) are statistically similar using DMRT ($p \leq 0{,}05$).

However, resistance against this disease agent is complex and many variants of the disease agent may co-exist. Resistance to common bean blight is particularly complex as 26 quantitative resistance loci to common bean blight have been described so far. To date, transcriptomic studies after common bean blight infection have been very scarce and the molecular mechanisms underlying susceptibility or resistance are largely unknown (Foucher et al., 2020).

Increased resistance can be developed by selecting for horizontal rather than vertical resistance (Garcia-Espinosa, 1997; Muimui et al., 2011). These results provide a basis to further understand the complex inheritance of common bean blight resistance in Mesoamerican common beans (Ambachew et al., 2021). These are just a few direct quotations to encourage researchers to breed for resistance to this disease.

In disease-complex situations, the tolerance/resistance to bean blight may be negligible. ISTA (2007), Karavina et al. (2011) and Chen et al. (2021) recommended an integrated disease management approach, which should include quarantine, cultural practices, and resistant varieties.

Trutmann et al. (1993) pointed out the efficacy of manipulating the microclimate in small fields as a viable measure to control this blight. Integrated management of this pathogen will likely be the trump card in the arsenal of farmers. This research is ongoing in the field to include elements of integrated management of common bean blight.

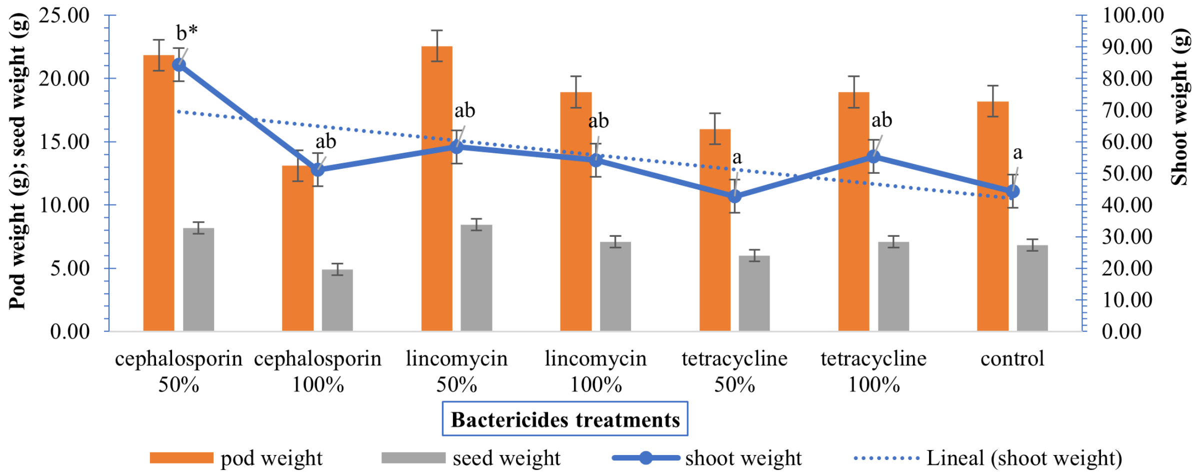


**Figure 6.** Effects of *Xanthomonas sp.* on shoot weight per plant, pod weight per plant, and seed weight per plant under the influence of bactericides in the screen-house. *Means overshadowed by the same letter(s) are statistically similar using DMRT ($p \leq 0{,}05$).

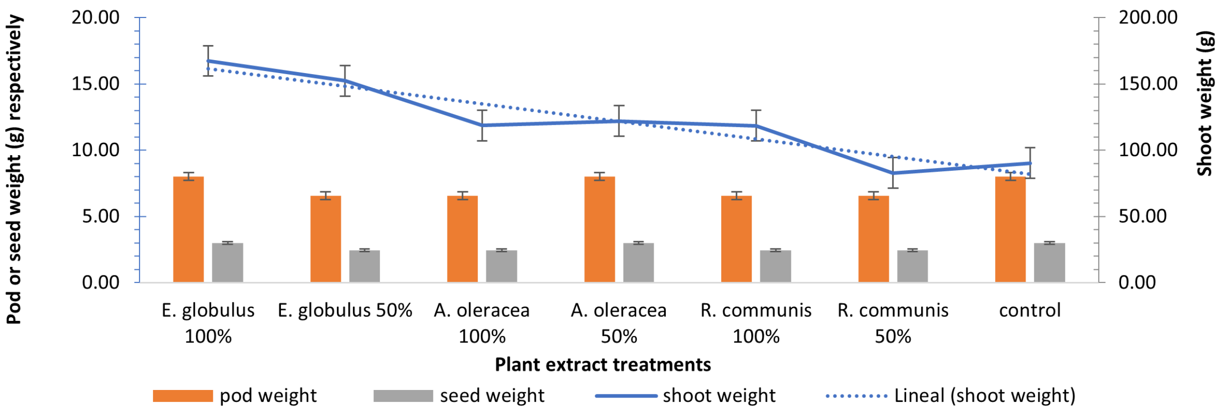


**Figure 7.** Effects of *Xanthomonas sp.* on shoot weight per plant, pod weight per plant, and seed weight per plant under the influence of plant extracts in the screen-house.

**Figure 8.** Symptoms of bean blight during the trial. **Top**: left = infected leaf in screen house. Middle = very early symptoms in screen house. Right = dying seedling in field. **Bottom**: left and middle = symptomatic and healthy plants. Right = highly infected or death seedlings.

## 4 Conclusions

Delving into the power of plant extracts and bactericidal agents applied against *Xanthomonas axonopodis* (the plant pathogenic bacterium behind bean blight disease) revealed that the bacteria could be managed effectively using botanicals and bactericides. Lincomycin, erythromycin, cephalosporin, and tetracycline were very effective against the pathogen. Likewise, *E. globulus*, *A. oleracea*, *A. melegueta* and *R. communis* also prevailed against the pathogen.

The synthetic antibiotics were better than the plant extracts at all times. Healthy beans could be produced using these plant extracts and bactericides. Meanwhile, work will continue on the availability, formulation, safety, and integration of these bacteria management agents for sustainable bean production. This study was carried out without hassles in the laboratory, in the screen-house the work became much difficult to carry out without contamination. In the field the work will be most difficult, hence we will need to obtain standard facilities to continue the work.

## Authors' contribution

E.M.N.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Research, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing– original draft, Writing– review and editing.

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Scientific paper/ Artículo científico
Agricultural Sciences

# Choice of trap plant and substrate for Mycorrhizal Inoculum production

## Elección de planta trampa y sustrato para la producción de Inóculo Micorrízico

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**Abstract**

Arbuscular mycorrhizal trap plants can be cultivated or wild species. In addition to withstanding anthropogenic pressure, these are excellent hosts for massive multiplication of arbuscular mycorrhizae. The objective of this work is to select the most suitable trap plant and substrate for the massive propagation of arbuscular mycorrhizal fungi. Four species were evaluated (*Cajanus cajan*, *Cynodon dactylon*, *Tagetes patula*, and *Plectranthus tomentosa*), two types of substrates (Substrate 1: sand, rice husk and vermiculite; Substrate 2: sand, rice husk and peat) and two phosphate sources (tricalcium phosphate and rock phosphate). At 120 days after inoculation, the percentage of mycorrhization and sporulation was evaluated. As a result, it was identified that the species *Plectranthus tomentosa* in substrate 2 was the most suitable, since it obtained a total mycorrhization of 79.7% at a concentration of 1000 ppm of tricalcium phosphate, while in substrate 1 it had 67.5% at the same concentration of tricalcium phosphate. This species also presented a higher number of spores (638 spore / 100 g soil) in substrate 1 at a concentration of 1000 ppm of tricalcium phosphate. In conclusion, the trap plant and substrate composition had a direct influence on the production of mycorrhizal inoculum.

***Keywords***: Endomycorrhizae, Sporulation, Host, Mycorrhization, Substrates.

**Resumen**

Las plantas trampas de micorrizas arbusculares pueden ser especies cultivables o silvestres. Además de soportar la presión antropogénica, éstas son excelentes huéspedes para la multiplicación masiva de las micorrizas arbusculares. El objetivo de este trabajo es seleccionar la planta trampa y el sustrato para la propagación masiva de micorrizas arbusculares. Se evaluaron cuatro especies (*Cajanus cajan, Cynodon dactylon, Tagetes patula,* y *Plectranthus tomentosa*), dos tipos de sustratos (Sustrato 1: arena, cascarilla de arroz y vermiculita; Sustrato 2: arena, cascarilla de arroz y turba) y dos fuentes fosfatadas (fosfato tricálcico y roca fosfórica). A los 120 días después de la inoculación se evaluó el porcentaje micorrización y esporulación. Como resultado se identificó que la especie *Plectranthus tomentosa* en el sustrato 2 fue la más idónea, ya que obtuvo una micorrización total de 79,7 % a una concentración de 1000 ppm de fosfato tricálcico, mientras que en el sustrato 1 tuvo 67,5 % a la misma concentración de fosfato tricálcico. Esta especie también presentó un mayor número de esporas (638 esporas / 100 g suelo) en el sustrato 1 a una concentración de 1000 ppm de fosfato tricálcico. En conclusión, la planta trampa y composición del sustrato tiene influencia directa en la producción de inóculo micorrízico.

***Palabras clave***: Endomicorrizas, Esporulación, Huésped, Micorrización, Sustratos.

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# 1 Introduction

Trap plants, environmental characteristics, and limited dispersion are factors that influence the mass multiplication of arbuscular mycorrhizal fungi (AMF) (Ramalho da Silva et al., 2014). The symbiotic associations between AMF communities and trap plants are complex, as mycorrhizal hyphal networks connect various rhizosphere systems and regulate nutrient flows, communication, and competitive interactions within the ecological niches of each plant. Coexistence with the plant community has led to interspecific specializations, such as the establishment of seedlings in new territories (Tedersoo et al., 2020; Van Geel et al., 2018).

Under natural conditions, most plants are colonized by AMF; however, the presence of these fungi can be affected by anthropogenic factors such as the excessive use of fertilizers, fungicides, and herbicides, leading to a decline or even disappearance of mycorrhizal biodiversity in agroecosystems (Davison et al., 2020). AMF are highly relevant in agriculture due to the multiple benefits they provide to plants, including phosphorus solubilization in the soil and the sustainability of agricultural production systems (Deepika and Kothamasi, 2015).

Trap plants serve as efficient hosts for AMF. One promising host plant is maize (*Zea mays* L.), as it allows for the production of one or more AMF species. Other plant species have also been used as trap plants to propagate AMF spores; these species belong to families such as *Solanaceae, Fabaceae, Cucurbitaceae, Amaryllidaceae,* and *Lamiaceae*, among others (Koske and Gemma, 1995; Salas and Blanco, 2000). Their biological cycles facilitate adaptation to the pot cultivation method, reducing production times to as little as 1.5 months (Salas and Blanco, 2000).

Leek (*Allium ampeloprasum* L.) is an ineffective species for inoculum production from AMF, as its performance may be influenced by environmental conditions. This suggests the hypothesis that plant diversity both stimulates and hinders spore production (Liu and Wang, 2003). The selection of trap plants is primarily based on the quantification of spore production per gram of soil or reference substrate. A limiting factor in spore production is the level of phosphorus (P) added to the substrate, as this element can interfere with spore formation in plants such as chili pepper (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.), leek (*Allium ampeloprasum* L.), and French marigold (*Tagetes patula* L.).

Other limiting factors associated with AMF propagation include the initial plant material, climatic factors, and the genetic diversity of AMF in the substrate composition (Koske and Gemma, 1995; Salas and Blanco, 2000; Schmidt et al., 2010). The substrate composition also influences the multiplication of mycorrhizal inoculum, particularly in the absorption of nutrients by the trap plant, such as P, Mg, and Ca (Gao et al., 2019). In particular, phosphorus dosage in the nutrient source has a differential effect on the benefits provided by AMF (Alarcón, 2003), impacting spore production efficiency in trap plants due to the origin of the phosphate source. Phosphate fertilization can either enhance or reduce the endogenous potential for mycorrhizal colonization (Covacevich et al., 2006).

For this reason, the objective of this study is to select a suitable trap plant and an optimal substrate for the mass propagation of arbuscular mycorrhizal fungi.

# 2 Materials and Methods

## 2.1 Systems conditions

This research was conducted in the greenhouse of the Biotechnology Research Center of Ecuador (CIBE) at the Escuela Superior Politécnica del Litoral (ESPOL), located at km 30.5 of the Perimeter Road on the Gustavo Galindo Campus (Latitude: 2° 9'3.12" S, Longitude: 79°57'13.03" W).

The average climatic conditions recorded in the greenhouse during the study period were 26 °C temperature, 65% relative humidity, and constant luminosity between May and August. The plant material used as trap plants included *Cajanus cajan, Cynodon dactylon, Tagetes patula,* and *Plectranthus tomentosa*, all obtained from wild seeds, except for *P. tomentosa*, which was propagated using 10 cm cuttings. To obtain homogeneous plants for the experiment, seedlings were initially grown in plastic seed trays. Once the seedlings developed two true leaves, they were transplanted into 2 kg polypropylene pots filled with different substrates according to the treatments and inoculated with 20 g of a native AMF consortium (purified native inoculum), comprising *Acaulospora sp., Ambispora sp., Diversispora sp., Entrophospora sp., Funeliformis sp.,* and *Glomus sp.* at a concentration of 300 total spores per 100 g of substrate (50 spores per genus). Notably, the AMF inoculum was obtained from the CIBE–ESPOL arbuscular mycorrhizal fungi (AMF) bank.

The study was designed using a two-factor experimental design, where Factor A (phosphate source and substrate) included four phosphate concentration levels, and Factor B consisted of the four plant species studied. The substrates used were: (S1) Sand, rice husk, and vermiculite, (S2) Sand, rice husk, and peat. The phosphate sources tested were tricalcium phosphate (TP) and phosphate rock (PR). The four plant species examined in this experiment were *C. cajan*, *C. dactylon*, *T. patula*, and *P. tomentosa*. Phosphate treatments were applied at three doses (150, 500, and 1000 ppm), along with a control (0 ppm), with three replicates per treatment. All substrates and materials were strictly sterilized before the experiment to prevent cross-contamination with pathogenic agents.

Agronomic parameters were measured to assess species selection attributes and physiological root mass characteristics, including plant height, leaf number, dry biomass, chlorophyll content index, mycorrhization percentage, and spore count at 120 days post-inoculation. In addition to phosphate fertilization, the plants were supplemented with 10 mL of modified Steiner solution, applied three times per week (Galindo Pardo et al., 2014).

## 2.2 Measurement of agronomic and physiological parameters

In this study, the following parameters were measured: plant height, recorded from the plant collar to the terminal bud; number of leaves emitted by each species, counted up to the last fully extended leaf; total dry biomass, determined using an oven at a temperature of 80 °C until a constant weight was obtained (López-Hidalgo et al., 2018); chlorophyll content index, analyzed at 120 days, for which the third youngest fully developed leaf was selected, and the measurement was taken between 10:00 and 11:00 AM, under optimal luminosity conditions, using a CCM-200 PLUS meter, Opti-Science (Redha et al., 2019).

To measure the total mycorrhization percentage in the roots of the trap plants, they were subjected to water stress for one week, during which irrigation and fertilization were suspended. After this period, a combined sample of 10 g of secondary roots from the three replicates of each treatment was collected. The roots were washed with potable water to remove impurities, then cut and placed in an amber container, applying 1 mL of 10% KOH for 10 minutes in an autoclave (125 °C and 15 psi pressure). After this time, the KOH was removed, washed with water, and 1% HCl was applied for 3 minutes to acidify the samples. The HCl was then removed without washing the roots, and finally 0.05% trypan blue was applied and sterilized for 15 minutes. Once sterilized, the samples were allowed to cool, and 10 rootlets were placed on each microscope slide. A drop of lactoglycerol was then added, and a cover slip was placed to observe the infective structures and determine the mycorrhization percentage (McGonigle et al., 1990).

For spore count determination, a 100 g substrate sample was taken in triplicate from each treatment. The samples were evaluated using the wet sieving method, where sieves of 45, 106, and 710 $\mu$m were used, and then centrifuged in a tween 20 plus sucrose gradient for 5 min at 2000 rpm (Gerderman and Nicholson, 1963). The final supernatant was dissolved in water to wash the spores (Furlan et al., 1980). The final content was poured into a segmented Petri dish where counting was performed using a stereoscope. The criterion for determining spore density across all combined species was the same: low density corresponds to $< 1$ spore/g of substrate; medium density corresponds to 1 – 10 spores/g of substrate; and high density corresponds to $> 10$ spores/g of substrate (Sieverding, 1983).

## 2.3 Statistical Analysis

The results were subjected to an analysis of variance (ANOVA) and Tukey's test with p-values $< 0.05$ for the studied variables, using the Infostat V.1.2.0 software (2017).

# 3 Results and Discussion

## 3.1 Growth parameters of trap plants with tricalcium phosphate (TF) and rock phosphate (RF) phosphate sources

Regarding plant height, in *C. cajan* plants, it was observed that the interaction between S1 and the phosphate source at 1000 ppm of FT resulted in heights of 79 cm, compared to the control, which reached 59 cm (Table 1). Meanwhile, at the same concentration in S2, the height of *C. cajan* was 59 cm, compared to the control, which reached 51 cm. With the RF phosphate source, plants reached a height of 71 cm at the maximum concentration, compared to 67 cm in the control in S1. In this substrate, 47 leaves were recorded, compared to 41 leaves in the control (Table 1). Similarly, a comparable pattern was observed in S2. Regarding the number of leaves in S1, a count of 51 leaves was recorded at a concentration of 1000 ppm of FT.

In *C. dactylon* plants, both S1 and S2 treatments at 1000 ppm of FT resulted in a height of 25 cm. The highest leaf count was observed in S2, where 50 leaves were obtained at 1000 ppm of FT, compared to 73 leaves in the control in S2. In S1, a chlorophyll content index (CCI) of 13 units was recorded at 1000 ppm of RF, compared to the control, which had 5 CCI units. For this species, no significant differences were observed in the evaluated variables in S1 and S2.

In *T. patula* plants, those grown in S1 reached a height of 6 cm in the 1000 ppm FT treatment, compared to 8 cm in the control. It is worth mentioning that this species showed minimal variation in S2, maintaining a pattern similar to the other species studied. On the other hand, in the 1000 ppm RF treatment, the height reached 9 cm, compared to 11 cm in the control in S1, while in S1 and S2, differences were not significant. The species *P. tomentosa* reached a height of 12 cm in S2 under the 1000 ppm RF treatment, compared to 17 cm in the control (Table 1).

Regarding chlorophyll content, no significant differences were observed in *C. cajan*, *T. patula*, and *P. tomentosa* in either substrate (Table 1). However, *C. dactylon* in S1 had 14 CCI units in the 1000 ppm FT treatment, compared to 7 CCI units in the control. *T. patula* plants in S2 recorded 11 CCI units in the 1000 ppm FT treatment, compared to 13 CCI units in the control. No other species exhibited statistically significant differences in either substrate, indicating that these species better adapt to the substrates and can maintain higher photosynthetic capacity and tolerance to water stress.

The experimental data revealed significant differences in plant growth with S2, which showed the highest adaptability across nearly all evaluated plant species. S1 had the greatest influence on *P. tomentosa* plants. These results indicate that trap plant selection plays a fundamental role in the production of mycorrhizal inoculum. Recent studies have demonstrated that the trap plant plays a crucial role in assembling the mycorrhizal community present in the inoculum (Van Geel et al., 2018), as plant species and fungal communities from specific sites enhance ecological restoration (Wu et al., 2020).

## 3.2 Mycorrhization of Trap Plants

The highest mycorrhization in S1 with both phosphate sources was observed in *P. tomentosa* plants, with mycorrhization levels exceeding 60 %, while the lowest mycorrhization was found in *C. cajan*, reaching approximately 25 % for both phosphate sources (Table 2). The species *C. dactylon* and *T. patula* showed similar percentages, with notable increases at 150 ppm of FT and RF, where both species achieved higher mycorrhization values.

In S2, *P. tomentosa* exhibited the highest mycorrhization percentage, reaching 71 % and 74 % in the 1000 ppm treatment with FT and RF, respectively, compared to the controls, which reached 68 % and 63 %. This indicates a higher affinity of the mycorrhizal consortium for the selected substrate and plant species compared to the other studied plant species.

Several reports have indicated that phosphate sources may affect the mycorrhizae in the initial inoculum. In this regard, *P. tomentosa* was the species that responded best to the highest phosphate source concentrations (FT and RF), which may be due to P availability influenced by pH (Lu et al., 2019). P absorption patterns can vary depending on its availability in the substrate or soil (Stewart et al., 2005).

Our results demonstrate that RF provides P and other essential elements for both plants and the mycorrhizal community, as these interactions are always modulated by the trap plant habitat conditions (Hu et al., 2019; Li et al., 2018, 2020). In the substrate, relative abundance changes occur in certain AMF species (Gigasporaceae, Glomeraceae, Diversisporaceae, and Acaulosporaceae), which can be attributed to nutrient fertility in the substrate's micro-ecosystem for continuous monitoring (Xiang et al., 2016).

**Table 1.** Growth of trap plants in substrates S1 and S2 and phosphate sources FT and RF (at concentrations of 0, 150, 500, and 1000 ppm): height (cm), number of leaves, chlorophyll content (CCI units).

| Trap plants | Treatment (ppm) | S1 Height (cm) | S1 Number of leaves | S1 Chlorophyll (U/CCI) | S2 Height (cm) | S2 Number of leaves | S2 Chlorophyll (U/CCI) |
|---|---|---|---|---|---|---|---|
| **Tricalcium Phosphate (FT)** | | | | | | | |
| *Cajanus cajan* | 0 (control) | *59 ± 6.5 $^a$ | *36 ± 1.8 $^a$ | 44 ± 1.1 $^a$ | 51 ± 1.7 $^a$ | 35 ± 0.5 $^a$ | 47 ± 2.2 $^a$ |
| | 150 | *66 ± 1.4 $^b$ | *43 ± 1.0 $^b$ | 45 ± 0.7 $^a$ | 59 ± 4.4 $^a$ | 40 ± 2.9 $^a$ | 51 ± 1.4 $^a$ |
| | 500 | *61 ± 4.3 $^b$ | *46 ± 2.7 $^b$ | 39 ± 3.0 $^a$ | 56 ± 2.0 $^a$ | 38 ± 3.4 $^a$ | 44 ± 3.9 $^a$ |
| | 1000 | *79 ± 1.4 $^b$ | *51 ± 2.3 $^b$ | 48 ± 4.1 $^a$ | 59 ± 1.5 $^a$ | 39 ± 3.2 $^a$ | 51 ± 0.9 $^a$ |
| *Cynodon dactylon* | 0 (control) | 23 ± 1.7 $^a$ | 65 ± 4.0 $^a$ | *7 ± 0.3 $^b$ | 27 ± 1.2 $^a$ | *73 ± 0.3 $^b$ | 9 ± 0.8 $^a$ |
| | 150 | 19 ± 1.2 $^a$ | 55 ± 3.7 $^a$ | *6 ± 1.8 $^a$ | 28 ± 1.1 $^a$ | *64 ± 8.0 $^b$ | 19 ± 5.1 $^a$ |
| | 500 | 23 ± 2.0 $^a$ | 61 ± 3.6 $^a$ | *14 ± 3.6 $^b$ | 26 ± 0.6 $^a$ | *51 ± 2.6 $^a$ | 16 ± 1.0 $^a$ |
| | 1000 | 25 ± 1.1 $^a$ | 63 ± 5.3 $^a$ | *14 ± 4.8 $^b$ | 25 ± 1.0 $^a$ | *50 ± 3.4 $^a$ | 15 ± 0.5 $^a$ |
| *Tagetes patula* | 0 (control) | 8 ± 0.5 $^a$ | 22 ± 1.1 $^a$ | 12 ± 0.9 $^a$ | 9 ± 0.3 $^a$ | 25 ± 2.0 $^a$ | *13 ± 0.0 $^b$ |
| | 150 | 8 ± 1.0 $^a$ | 22 ± 1.3 $^a$ | 14 ± 4.0 $^a$ | 10 ± 0.5 $^a$ | 22 ± 0.6 $^a$ | *11 ± 0.2 $^b$ |
| | 500 | 7 ± 1.8 $^a$ | 20 ± 0.6 $^a$ | 16 ± 5.0 $^a$ | 8 ± 0.6 $^a$ | 23 ± 1.3 $^a$ | *12 ± 1.1 $^b$ |
| | 1000 | 6 ± 0.0 $^a$ | 20 ± 0.6 $^a$ | 19 ± 3.1 $^a$ | 9 ± 0.5 $^a$ | 24 ± 1.1 $^a$ | *11 ± 1.1 $^a$ |
| *Plectranthus tomentosa* | 0 (control) | 13 ± 2.0 $^a$ | 64 ± 2.4 $^a$ | 13 ± 2.8 $^a$ | 14 ± 1.1 $^a$ | *70 ± 0.0 $^a$ | 15 ± 4.4 $^a$ |
| | 150 | 13 ± 1.7 $^a$ | 68 ± 4.3 $^a$ | 16 ± 0.3 $^a$ | 11 ± 1.1 $^a$ | *74 ± 0.6 $^b$ | 17 ± 0.5 $^a$ |
| | 500 | 9 ± 0.6 $^a$ | 58 ± 4.0 $^a$ | 11 ± 1.9 $^a$ | 11 ± 0.8 $^a$ | *80 ± 1.7 $^c$ | 15 ± 3.6 $^a$ |
| | 1000 | 12 ± 2.0 $^a$ | 60 ± 7.6 $^a$ | 15 ± 2.9 $^a$ | 12 ± 0.5 $^a$ | *76 ± 0.8 $^c$ | 18 ± 0.1 $^a$ |
| **Phosphate Rock (RF)** | | | | | | | |
| *Cajanus cajan* | 0 (control) | 67 ± 1.1 $^a$ | 41 ± 1.2 $^a$ | 44 ± 1.1 $^a$ | 63 ± 4.3 $^a$ | 41 ± 1.2 $^a$ | 46 ± 1.1 $^a$ |
| | 150 | 60 ± 1.8 $^a$ | 42 ± 1.4 $^a$ | 45 ± 0.9 $^a$ | 52 ± 0.6 $^a$ | 36 ± 1.4 $^a$ | 46 ± 1.0 $^a$ |
| | 500 | 58 ± 6.0 $^a$ | 43 ± 4.5 $^a$ | 43 ± 1.7 $^a$ | 65 ± 2.5 $^a$ | 42 ± 4.5 $^a$ | 49 ± 2.0 $^a$ |
| | 1000 | 71 ± 4.9 $^a$ | 47 ± 3.7 $^a$ | 42 ± 1.3 $^a$ | 61 ± 0.3 $^a$ | 41 ± 3.7 $^a$ | 50 ± 0.4 $^a$ |
| *Cynodon dactylon* | 0 (control) | 20 ± 1.7 $^a$ | *68 ± 4.0 $^a$ | *5 ± 1.1 $^a$ | 30 ± 0.0 $^a$ | 67 ± 2.6 $^a$ | 13 ± 0.2 $^a$ |
| | 150 | 23 ± 1.2 $^a$ | *49 ± 0.6 $^a$ | *20 ± 2.7 $^b$ | 28 ± 0.6 $^a$ | 64 ± 2.6 $^a$ | 21 ± 8.7 $^a$ |
| | 500 | 22 ± 0.3 $^a$ | *44 ± 0.8 $^b$ | *13 ± 2.0 $^b$ | 27 ± 1.3 $^a$ | 68 ± 4.9 $^a$ | 19 ± 3.1 $^a$ |
| | 1000 | 22 ± 2.0 $^a$ | *63 ± 2.6 $^b$ | *13 ± 1.7 $^b$ | 28 ± 1.0 $^a$ | 59 ± 6.2 $^a$ | 16 ± 2.5 $^a$ |
| *Tagetes patula* | 0 (control) | 11 ± 2.0 $^a$ | 21 ± 1.7 $^a$ | 18 ± 1.9 $^a$ | 9 ± 0.6 $^a$ | 20 ± 0.6 $^a$ | *14 ± 0.8 $^b$ |
| | 150 | 11 ± 0.8 $^a$ | 20 ± 1.1 $^a$ | 14 ± 1,0 $^a$ | 9 ± 0.5 $^a$ | 22 ± 1.7 $^a$ | *13 ± 0.9 $^b$ |
| | 500 | 10 ± 3.1 $^a$ | 22 ± 1.1 $^a$ | 16 ± 1.6 $^a$ | 8 ± 1.0 $^a$ | 22 ± 1.1 $^a$ | *12 ± 0.5 $^b$ |
| | 1000 | 9 ± 3.0 $^a$ | 22 ± 1.3 $^a$ | 17 ± 1.7 $^a$ | 9 ± 1.3 $^a$ | 24 ± 1.3 $^a$ | *15 ± 0.4 $^b$ |
| *Plectranthus tomentosa* | 0 (control) | 13 ± 1.2 $^a$ | 66 ± 4.0 $^a$ | 12 ± 1.3 $^a$ | *17 ± 0.8 $^b$ | *73 ± 1.6 $^a$ | 18 ± 2.2 $^a$ |
| | 150 | 10 ± 1.2 $^a$ | 59 ± 3.3 $^a$ | 12 ± 4.0 $^a$ | *13 ± 0.8 $^a$ | *79 ± 2.4 $^b$ | 18 ± 2.5 $^a$ |
| | 500 | 10 ± 0.3 $^a$ | 63 ± 6.6 $^a$ | 9 ± 1.1 $^a$ | *15 ± 1.1 $^b$ | *84 ± 0.6 $^b$ | 15 ± 1.3 $^a$ |
| | 1000 | 10 ± 1.0 $^a$ | 58 ± 3.0 $^a$ | 7 ± 1.5 $^a$ | *12 ± 0.5 $^a$ | *72 ± 3.0 $^a$ | 18 ± 0.9 $^a$ |

Substrates: S1 = sand, rice husk, and vermiculite; S2 = sand, rice husk, and peat; FT = Tricalcium phosphate; RF = Phosphate rock. Mean values ± standard error, comparisons are made according to the phosphate source used. *Different letters in the same row indicate significant differences according to Tukey's test ($p < 0.05$).

**Table 2.** Mycorrhization percentages in trap plant roots, in substrates S1 and S2, and phosphate sources FT and RF (at concentrations of 0, 150, 500, and 1000 ppm).

| Percentage of mycorrhization (%) | | | | | |
|---|---|---|---|---|---|
| Trap plants | Treatment (ppm) | FT | | RF | |
| | | S1 | S2 | S1 | S2 |
| *Cajanus cajan* | 0 | 27± 2.5 $^{c}$ | 25±2.8 $^{c}$ | 35±1.3 $^{f}$ | 33±2.5 $^{d}$ |
| | 150 | 25±1.7 $^{b}$ | 32±10 $^{d}$ | 41±3.5 $^{g}$ | 18±0.6 $^{a}$ |
| | 500 | 31±2.0 $^{e}$ | 23±1.3 $^{b}$ | 29±2.2 $^{d}$ | 31±3.3 $^{d}$ |
| | 1000 | 22±0.9 $^{a}$ | 31±4.2 $^{d}$ | 24±0.7 $^{b}$ | 32±0.7 $^{d}$ |
| *Cynodon dactylon* | 0 | 39±1.9 $^{f}$ | 39±2.7 $^{d}$ | 42±1.7 $^{g}$ | 38±0.0 $^{e}$ |
| | 150 | 46±3.6 $^{i}$ | 47±2.4 $^{e}$ | 43±1.7 $^{c}$ | 34±2.8 $^{d}$ |
| | 500 | 42±1.3 $^{g}$ | 34±2.8 $^{d}$ | 34±2.4 $^{f}$ | 42±3.2 $^{e}$ |
| | 1000 | 45±2.4 $^{h}$ | 31±4.2 $^{e}$ | 38±2.6 $^{f}$ | 48±0.7 $^{f}$ |
| *Tagetes patula* | 0 | 63±1.7 $^{l}$ | 42±1.1 $^{g}$ | 60±1.8 $^{l}$ | 51±0.0 $^{e}$ |
| | 150 | 71±3.2 $^{l}$ | 50±1.6 $^{f}$ | 62±5.2 $^{l}$ | 45±2.0 $^{e}$ |
| | 500 | 45±5.4 $^{h}$ | 45±1.3 $^{e}$ | 55±3.5 $^{k}$ | 45±3.8 $^{e}$ |
| | 1000 | 46±5.5 $^{i}$ | 46±1.8 $^{e}$ | 49±1.9 $^{j}$ | 40±4.0 $^{e}$ |
| *Plectranthus tomentosa* | 0 | 68±4.9 $^{l}$ | 68±3.1 $^{h}$ | 67±2.4 $^{l}$ | 63±0.6 $^{h}$ |
| | 150 | 63±1.3 $^{l}$ | 79±1.9 $^{h}$ | 61±1.6 $^{l}$ | 75±2.4 $^{h}$ |
| | 500 | 65±3.5 $^{l}$ | 77±2.5 $^{h}$ | 67±2.6 $^{l}$ | 79±3.1 $^{h}$ |
| | 1000 | 69±1.3 $^{l}$ | 71±6.1 $^{h}$ | 69±4.2 $^{l}$ | 74±1.0 $^{h}$ |

Substrates: S1 = sand, rice husk, and vermiculite; S2 = sand, rice husk, and peat; FT = tricalcium phosphate; RF = phosphate rock. Mean values ± standard error, comparisons are made according to the phosphate source used. *Different letters in the same row indicate significant differences according to Tukey's test ($p<0.05$)

## 3.3 Spore Production in the Substrates

This study found that different trap plants can adapt to a mycorrhizal inoculum and substrate composition, forming a specific nesting by affinity during the plant's life cycle. This was supported by the differences observed in spore production. The fact that we used several mycorrhiza-dependent trap plants and a native inoculum under water stress suggests that these factors may have significantly contributed to the synergistic effects in trap plant development.

In *P. tomentosa* plants grown in S1, the highest spore count was obtained, reaching 637 and 623 spores per 100 g of soil in FT and RF, respectively, at a 1000 ppm concentration, compared to the controls (T0), which reached 434 and 438 spores per 100 g of soil in FT and RF (Figure 1). On the other hand, the species with the lowest spore count was *C. cajan*, with 195 and 198 spores per 100 g of soil in FT and RF, respectively, at a 1000 ppm concentration, compared to T0, which reached 211 and 166 spores per 100 g of soil in FT and RF, respectively. The other species exhibited similar trends in treatments with 150 and 500 ppm of phosphate sources.

In *P. tomentosa* plants grown in S2, the highest spore count was obtained, reaching 612 and 623 spores per 100 g of soil in FT and RF, respectively, at a 1000 ppm concentration, compared to T0, which reached 426 and 506 spores per 100 g of soil in FT and RF (Figure 2).

The species with the lowest spore count was *C. cajan*, with 256 and 238 spores per 100 g of soil in FT and RF, respectively, at a 1000 ppm concentration, compared to T0, which reached 187 and 133 spores per 100 g of soil in FT and RF, respectively. The other species followed similar patterns in treatments with 150 and 500 ppm of phosphate sources.

The results demonstrate that *P. tomentosa* exhibited higher root mycorrhization by the fifth month, with mycorrhization levels exceeding 60 % and 70 % in FT and RF, respectively, where the infective structure characteristics of AMF were observed (Figure 3). This suggests an effective complementarity when selecting a trap plant for biotechnological applications or for restoring contaminated soils.

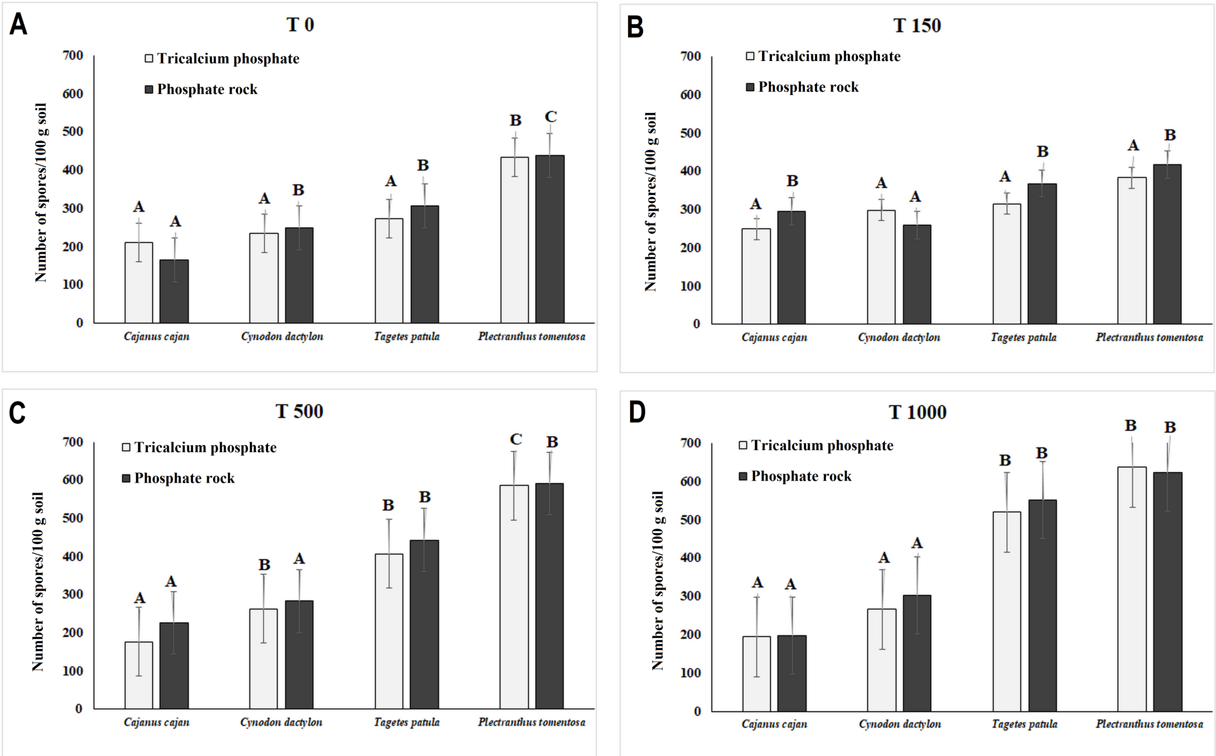


**Figure 1.** Spores produced in substrate 1; A) Treatment 0 (control), B) Treatment 150 ppm, C) Treatment 500 ppm, D) Treatment 1000 ppm. The bar limits represent the standard error with a 95 % confidence interval. Different letters above the bars indicate statistically significant differences according to Tukey's test ($p<0.05$).

Trap plants that experience multiple stress factors and are inoculated with different mycorrhizal species are more efficient than those not inoculated or those that have not been enhanced with a greater diversity of AMF genera (Crossay et al., 2019).

We found that plant height, chlorophyll content, and biomass in trap plants respond to the type of substrate used and the phosphate source (FT or RF). These results are consistent with reports indicating that mycorrhizal inoculation enhances photosynthesis, root expansion, and water and nutrient uptake (Selvakumar et al., 2018).

This information is crucial for trap plant selection criteria, as an acceptable biomass production performance may stimulate or delay mycorrhizal colonization, which in turn affects spore production (Liu and Wang, 2003).

The density of trap plants is also considered a limiting factor, as plants compete for inorganic P in the substrates, which could enhance spore production (Fabiańska et al., 2020), particularly in tillering plants, such as *C. dactylon*.

Regardless of nutrient variations, the AMF fungal network provides trap plants with a constant P supply, making them effective resource managers (Van't Padje et al., 2021b). Colonization patterns have shown that time and the nutrient requirements of the trap plant play a crucial role, as these factors are highly dynamic and difficult to predict in natural conditions. This represents a challenge in studying host-symbiont relationships that are measurable and reliable within the reality of natural associations (Van't Padje et al., 2021a).

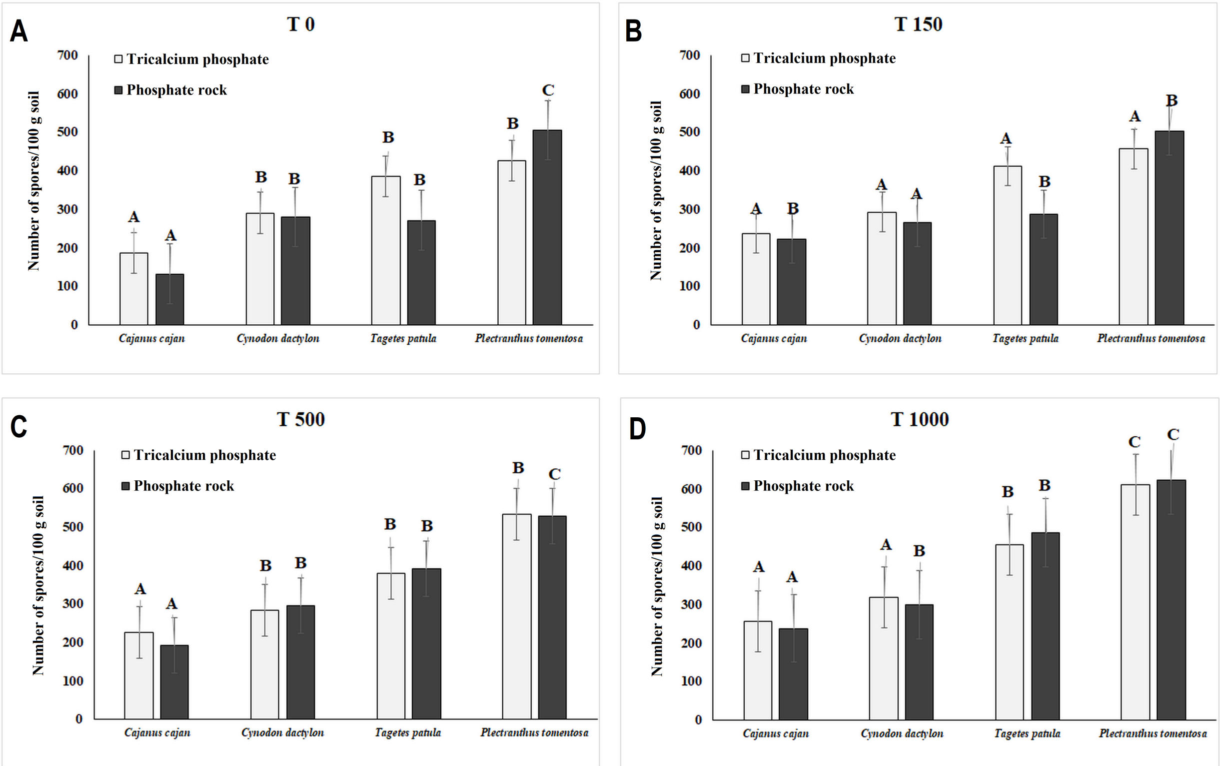


**Figure 2.** Spores produced in substrate 2; A) Treatment 0 (control), B) Treatment 150 ppm, C) Treatment 500 ppm, D) Treatment 1000 ppm. The bar limits represent the standard error with a 95 % confidence interval. Different letters above the bars indicate statistically significant differences according to Tukey's test ($p<0.05$).

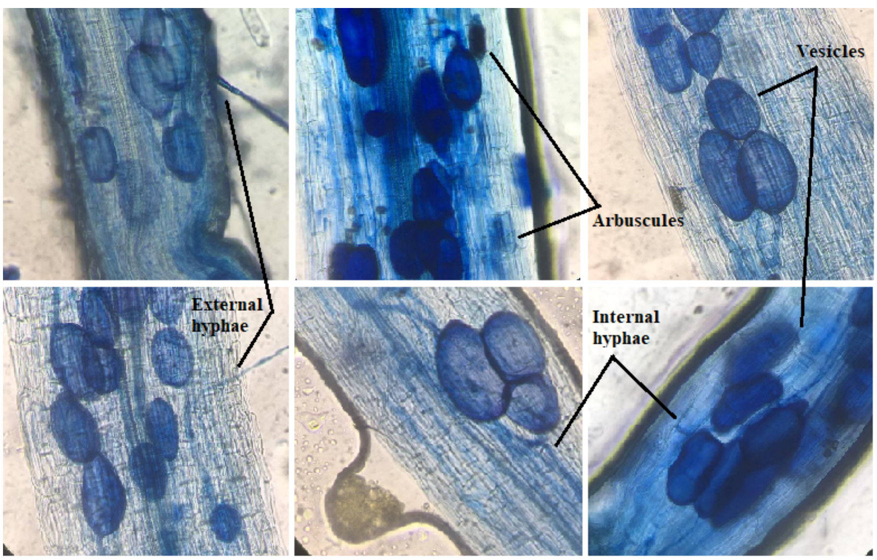


**Figure 3.** Mycorrhized roots of *P. tomentosa* observed under an optical microscope at 400X magnification, 120 days post-inoculation.

# 4 Conclusions

Among the four trap plants evaluated, the species that responded best to the mycorrhizal consortium was *Plectranthus tomentosa*. This species exhibits promising traits for mycorrhizal inoculum production, regardless of the substrate type used.

In contrast, *Cajanus cajan* was the least efficient host for AMF spore production.

The dosage of phosphate sources did not affect mycorrhizal colonization or spore production in the studied species, but it did impact their growth.

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## Author's contribution

J.N.M.: Conceptualization, Writing- original draft, Methodology. K.O.F.: Research, Data curation. R.O.A.: Writing- review editing, Formal analysis, Resources, Visualization. M.B.A.: Project administration, Supervision, Funding acquisition.

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Earth Sciences

# Effects of change in land use and climate change on the potential distribution of sugarcane at Valle del Chota, Ecuador

## Efectos del cambio de uso de suelo y cambio climático en la distribución potencial de la caña de azúcar en el Valle del Chota, Ecuador

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**Abstract**

Sugarcane is a socio-economically important crop, which is not exempt from internal and external disturbances. This research was carried out at the Chota Valley, one of the sectors where sugarcane is traditionally grown in Ecuador. The aim is to determine the effects of future land cover change on its development until 2031 and the effects of climate change on its distribution for the period 2025-2035 in the scenarios RCP 4.5 and RCP 8.5. For this purpose, land use was projected for 2022 and 2031, using land use coverages for 1999-2011 as the base information. Future projections under climate change scenarios were carried out using the agro ecological zoning of crops and changes in temperature and precipitation climate scenarios. The results forecast that due to land use change, sugarcane cultivation will decrease its area by about 14.65 % from 2022 to 2031. However, the research findings under the RCP 4.5 and RCP 8.5 climate scenarios for the 2025-2035 period indicate a significant increase in the optimal areas for sugarcane production in equal proportion, mirroring the rise in water availability.

***Keywords***: Sugarcane, Land use change, climate change, RCP, Chota.

**Resumen**

La caña de azúcar es un cultivo de importancia socioeconómica, el cual no está exento de perturbaciones internas y externas. La presente investigación se desarrolló en el Valle del Chota, que es uno de los sectores donde tradicionalmente se cultiva la caña de azúcar en Ecuador. El objetivo es determinar los efectos del cambio futuro de cobertura de suelo en su desarrollo hasta el año 2031 y los efectos del cambio climático en su distribución para el periodo 2025-2035 en los escenarios RCP 4,5 y RCP 8.5. Para ello, se proyectó el uso de suelo a los años 2022 y 2031 utilizando como información base las coberturas de uso de suelo de los años 1999 y 2011. Para la proyección futura bajo escenarios de cambio climático se utilizó la zonificación agroecológica del cultivo y los cambios que ocurrirán en la temperatura y precipitación en los escenarios climáticos. Los resultados pronostican que, por efecto del cambio de uso de suelo, el cultivo de caña de azúcar disminuirá su superficie en alrededor del 14,65% desde el año 2022 hasta el 2031. En contraste, bajo los escenarios climáticos RCP 4,5 y RCP 8,5, para el periodo 2025-2035 las superficies óptimas para la producción de caña de azúcar aumentarán en igual proporción, reflejando un aumento de la disponibilidad hídrica.

***Palabras clave***: Caña de azúcar, Cambio de uso de suelo, Cambio climático, RCP, Chota.

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# 1 Introduction

Sugarcane (*Sacharum officinarum*) is a perennial plant of the Poaceae family belonging to the *Saccharum*, genus, used primarily for sugar production. Sugarcane transformed the industrial business activity, shifting from being a food-providing crop to one that supplies inputs for the chemical and energy industries (Gómez-Merino et al., 2015). The economic importance of this crop lies in three main characteristics: (a) it has a high production capacity; (b) it efficiently utilizes productive resources and inputs; and (c) it can be locally processed into higher-value products such as sucrose, molasses, ethanol, and energy (Gómez-Merino et al., 2015). Currently, it is one of the most important crops worldwide, as its production contributes to the nourishment of over half of the global population (Moraes et al., 2015; Som-ard et al., 2018). Global production has increased from 448 million tons cultivated over 8.9 million hectares in 1961 to 2 billion tons over 27 million hectares in 2020 (El Chami et al., 2020).

Sugarcane originates from the tropical, warm-temperate regions of Southeast Asia and New Guinea and is primarily cultivated in tropical and subtropical regions worldwide (Som-ard et al., 2021). In Ecuador, sugarcane was introduced to tropical and subtropical zones during the Spanish conquest and currently it covers more than 82,000 hectares (Mendoza et al., 2005). The Chota Valley, located between the provinces of Carchi and Imbabura in Ecuador, is one of the subtropical areas with suitable edaphic conditions for cultivating this crop (Echeverría and Uribe, 1981). Sugarcane arrived in the Chota Valley with the first Spanish landowners around 1550; however, it was not until 1570 that sugarcane began to replace the primary crops of the time-coca and cotton (Coronel Feijoo, 1991). Sugarcane production has become one of the main sources of income for the population in this area, which is the second-largest Afro-descendant settlement in Ecuador. Nevertheless, this agricultural activity has not been immune to internal and external disturbances, such as crop replacement (Espín Díaz, 1999) or the potential effects of climate change.

Globally, agriculture is the economic sector most affected by climate fluctuations, making it essential to implement effective strategies for adaptation and mitigation (Zhao and Li, 2015). Climate change results from disruptions in the Earth's energy balance, caused by both natural factors and human interventions (Intergovernmental Panel On Climate Change (IPCC), 2023). This phenomenon presents new challenges for agricultural productivity, as it will eventually alter climatic and agroecological zones, particularly in regions such as Latin America (López Feldman and Hernández Cortés, 2016). According to Organización Internacional del Azúcar (2013), climate change by 2050 could increase the area suitable for sugarcane cultivation by 160 %. Changes in climatic and atmospheric conditions-such as temperature rise, solar radiation, and $CO_2$ concentration-are expected to increase sugarcane yields (Guerra and Hernández, 2012).

However, there is no global consensus on the potential local effects of climate change on sugarcane production. While Marin et al. (2013) and Todd et al. (2015) suggest that climate change could improve sugarcane yields in Brazil and Louisiana (USA), respectively-due to improved water use efficiency in Brazil and reduced frost incidence in Louisiana-Singh and El Maayar (1998) found that in the Southern Caribbean, sugarcane yields could decrease by 20–40 % under a scenario of doubled $CO_2$ concentration. Similarly, Knox et al. (2010) used climate models to predict a future decline in sugarcane yields in Swaziland, Africa.

These findings confirm that farmers must adapt to new conditions for sugarcane cultivation under the possible effects of climate change, whether positive or negative. In Ecuador, according to Representative Concentration Pathway (RCP) projections, national temperatures could rise by up to 2 °C, except in the Amazon and Insular regions (Ministerio de Ambiente de Ecuador, 2019). This temperature change would reduce the adaptive capacity of Ecuadorian cities to climate change (Arias-Muñoz et al., 2022), and inevitably affect the yields of crops such as sugarcane. Moreover, future sugarcane yields may not only be impacted by climate change but also by dynamics related to land-use change. Therefore, the aim of this study is to determine the current extent of sugarcane cultivation (in 2022) and project its area to the year 2031 under a baseline scenario with land-use change trends, and under two climate change scenarios (RCP 4.5 and RCP 8.5). Land-use changes were assessed using a transition matrix, and land-use projections, including sugarcane coverage, were developed using Markov chains.

Future projections under climate change scenarios were based on the crop's agroecological zoning and the anticipated impacts of temperature and precipitation on agroecological requirements. Thus, the challenge was to identify optimal agroecological zones, assess possible future changes and emerging needs, and propose adaptation strategies aimed at minimizing the impact of climate change to foster sustainable development (Oviedo and León, 2010).

# 2 Materials and Methods

## 2.1 Study area

The study area of this research corresponds to the dry valley of the Chota River, located in northern Ecuador between the provinces of Imbabura and Carchi, within the meridians 78° 15′ and 77° 55′ West longitude, and parallels 0° 30′ and 0° 7′ North latitude (Espín Díaz, 1999). It covers an area of 9,247 hectares and is characterized as an enclosed valley within the Chota basin, situated between two branches of the Andes mountain range (Winckell et al., 1997) The valley is also characterized by plains and peneplain relief, with the Chota River being the main watercourse traversing it (Figure 1).

The Chota Valley features a lower montane dry shrubland vegetation formation (Sierra, 1999) and is located at elevations ranging from 1,500 to 1,800 meters above sea level (Espín Díaz, 1999). According to Pourrut's climatic classification Pourrut (1983), the valley's climate is considered megathermal arid to semi-arid, with an average annual temperature ranging from 17.2 to 19.5 °C and an average annual precipitation between 559 and 945 mm. The characteristic vegetation of the area includes species such as *Acacia macracantha* and *Mimosa pudica* (algarrobo), *Mimosa quitensis* (huarango), *Spondias mombin* (ovo), *Phaseolus vulgaris* (common bean), *Cajanus cajan* (pigeon pea), and *Saccharum officinarum* (sugarcane) (Mena, 2001).

The population of the Chota Valley is predominantly Afro descendant. The Afro-Ecuadorian community that settled in the valley traces its origins to the importation of enslaved labor by the Jesuits for sugarcane plantations (Carrascal Jijón, 2016). This historical process gave rise to a culturally and historically distinct Afro-descendant community in the Chota Valley. In fact, it is considered the second-largest Afro-descendant settlement in Ecuador, surpassed only by the province of Esmeraldas (Ortiz Villalva, 2011). The valley comprises the following population centers: Piquiucho, Chalguayaco, Juncal, Pusir Grande, Pusir Chiquito, Carpuela, Tumbatú, Ambuquí, San Vicente de Pusir, Chota, and Mascarilla. Within these communities, it is estimated that approximately 64.4 % of the population lives in poverty, and as of 2001, around 15 % of the population was considered illiterate (Ortiz Villalva, 2011; Peralta et al., 2001).

The primary economic activities include agriculture (86 %), manufacturing (10 %), and commerce (4 %) (Peralta et al., 2001). However, agriculture in the region has faced significant challenges due to excessive land fragmentation, insufficient irrigation water, limited agricultural technology, and the introduction of new market demands (Ortiz Villalva, 2011). These conditions have led to population migration toward urban areas, continuous crop rotation, and even the pursuit of alternative economic activities (Espín Díaz, 1999).

On the other hand, in 1964, the Ingenio Azucarero del Norte (IANCEM) sugar mill was established to take advantage of the region's favorable conditions for sugarcane cultivation and to improve the economic conditions of local farmers (Espín Díaz, 1999). Nevertheless, the valley only briefly served as a sugarcane supplier for the mill, as local farmers found other crops-such as common beans (*Phaseolus vulgaris*), chili peppers (*Capsicum annuum*), and tomatoes (*Lycopersicon esculentum*)-to be more profitable in nearby local markets such as Pimampiro, Ibarra, and Tulcán (Espín Díaz, 1999).

## 2.2 Determination of the current (2022) and future expansion (2031) of sugarcane (*Saccharum officinarum*) cultivation

The current (2022) and projected (2031) distribution of sugarcane was determined based on land use projections for the Chota Valley, using data from the reference years 1999 and 2011, through the application of ArcGIS 10.8.2 and TerrSET 1.0 software. The projection of Land cover and Land Use Change (LULC) considered the following predictive variables: land slope, digital elevation model (DEM), Euclidean distance from urban areas, roads, and Euclidean distance from roads (Figure 2a–e).

The variables were classified as either static or dynamic, according to their characteristics. The following were defined as static variables: (a) the digital elevation model (DEM), and (b) slope, as these are elements that do not change over time. In contrast, the dynamic variables included: (c) Euclidean distance from populated areas, (d) Euclidean distance from roads, and (e) road networks.

Furthermore, for the land cover and land use (LULC) projection, as recommended by Ortega Chuquín and Arias Muñoz (2022), the spatial characteristics of the predictive variables-slope, altitude, distance to roads, and distance to urban areas-were standardized, along with the spatial data files corresponding to the reference years for land cover and land use (1999 and 2011). This process involved the harmonization of both the spatial and radiometric resolution of the geospatial datasets (Table 1).

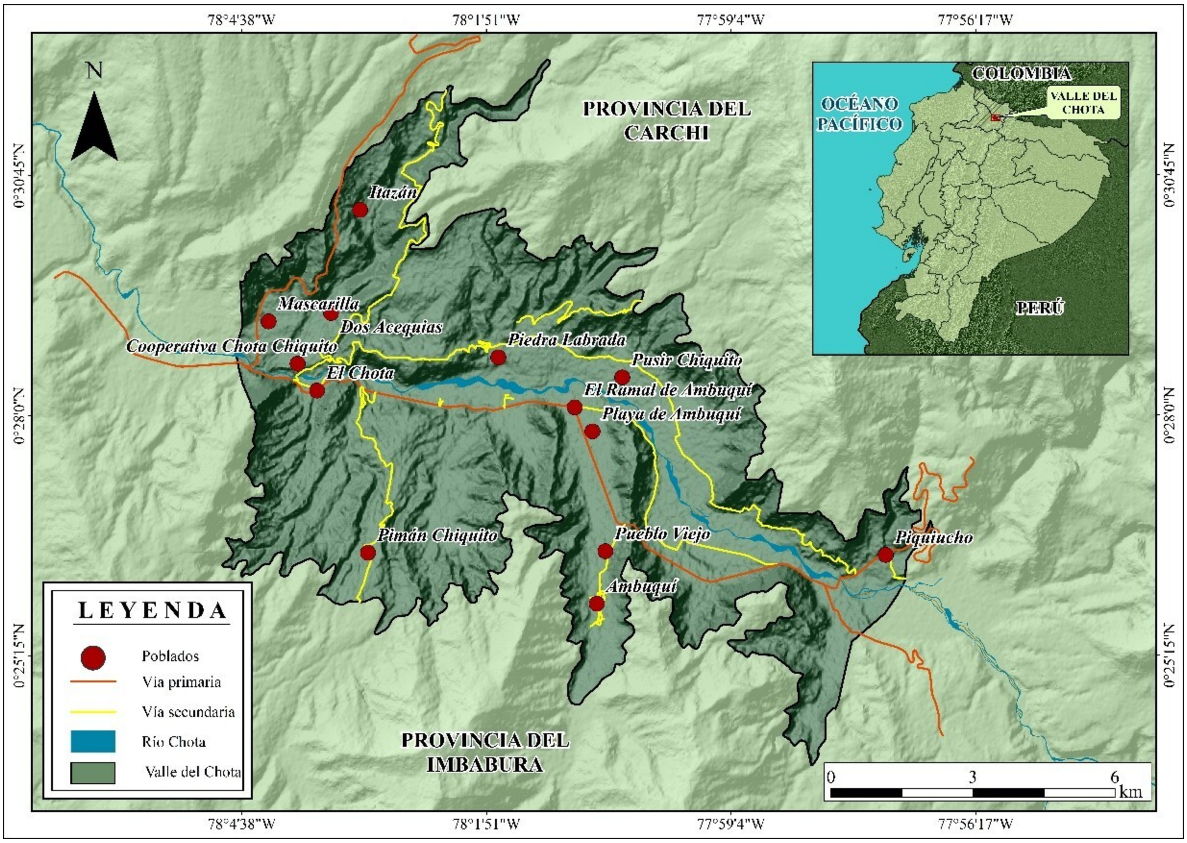


**Figure 1.** Location of the Chota Valley.

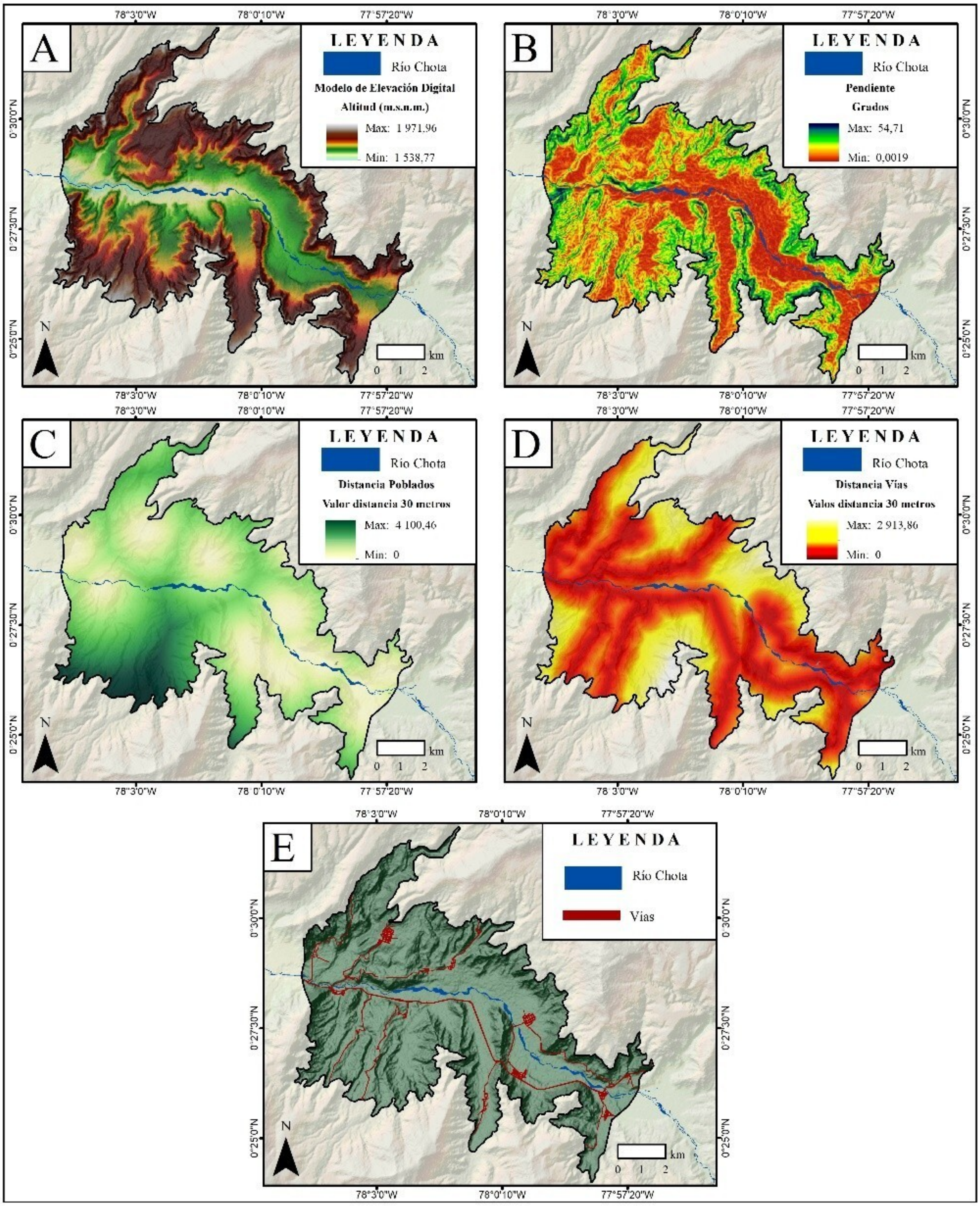


**Figure 2.** Static and dynamic variables: a) DEM and elevation model, b) slopes, c) Euclidean distance of settlements, d) Euclidean distance of roads and e) roads.

**Table 1.** Characteristics of geospatial files

| Raster information | Features |
|---|---|
| Columns and rows | 502-497 |
| Number of bands | 1 |
| Pixel size | 30 m × 30 m |
| Formato | TIFF |
| Pixel type | Unsigned integer |
| Radiometric resolution of the pixel | 4 bits |

To establish the land cover and land use (LULC) for the years 2022 and 2031, the Land Change Modeler (LCM) tool from the TerrSET 1.0 software was used. This enabled the development of potential transition models, prediction models, and land use change scenarios. In this study, a submodel titled "Disturbances"was developed, based on identifying the most predominant trends in land cover and land use change among the different categories (Table 2).

**Table 2.** Disturbance submodel

| Transition | | Sub - Model |
|---|---|---|
| **From:** | **Change to:** | |
| Sugarcane cultivation | Other crops | Disturbances |
| Xerophytic vegetation | Sugarcane | |
| Sugarcane cultivation | Non-vegetated area | |
| Other crops | Sugarcane | |

Once the submodel was established, Cramér's V test was applied to the previously generated static and dynamic variables. This test is used to measure the association between two categorical variables and it enables the calculation of the relationship between variables based on their effect (Table 3).

**Table 3.** Interpretation of Cramer's V test results

| Effect Size | Interpretation |
|---|---|
| $\leqslant 0.2$ | The result is weak. Although the result is statistically significant, the fields are only weakly associated. |
| $0.2 < ES \leqslant 0.6$ | The result is moderate. The fields are moderately associated. |
| $>0.6$ | The result is strong. The fields are strongly associated. |

Based on this interpretation of Cramér's V test, variables with moderate and strong levels of association were identified, as shown in Table 4, allowing for the development of the transition model for the years 2022 and 2031. Finally, to assess land cover and land use change for 2022 and 2031, the CA-Markov analysis was performed using TerrSet 1.0 software. This model is based on Markov chains, originally proposed by Russian mathematician Andrei Markov in 1907 (López Granados et al., 2001).

**Table 4.** Cramer's V test analysis

| Variables | Cramer's V Test |
|---|---|
| Slope | 0.546 |
| Elevation model | 0.573 |
| Euclidean distance from settlements | 0.457 |
| Roads | 0.321 |
| Euclidean distance from roads | 0.441 |
| Sugarcane cultivation to non-vegetated area | 0.393 |
| Sugarcane cultivation to other crops | 0.645 |
| Xerophytic vegetation to sugarcane cultivation | 0.498 |

## 2.3 Future sugarcane crop zoning under climate change scenarios to 2031

First, the agroecological requirements of the crop were identified and used to zone the optimal areas for the development of sugarcane (Table 5). Agroecological requirements help define suitable zones for crops based on combinations of soil, physiography, and climate (FAO, 1978).

Additionally, they contribute to the spatial zoning of crop distribution and the identification of potential impacts of climate change on the future viability of agriculture. Crops require optimal conditions for their development, which may include physical, chemical, topographic, and climatic factors-such as temperature and precipitation-that can limit the growth and development of the crop (Ruiz Corral et al., 2013).

**Table 5.** Agroecological conditions of sugarcane cultivation

| | |
|---|---|
| **Average annual temperature** | 15 to 33 °C |
| **Average annual precipitation** | 1200 a 1500 mm/year |
| **pH** | 5.5 to 8 |
| **Type of soil recommended** | Loam, clay loam or sandy-clay loam |

**Source:** Duarte Álvarez and González Villalba (2019)

Subsequently, the agroecological requirements for temperature and precipitation were replaced with the estimated values under the RCP 4.5 and RCP 8.5 scenarios. The climate projection data were obtained from the Climate Projections Report of Ecuador developed by the Ministry of the Environment, Water and Ecological Transition (Ministerio de Ambiente, Agua y Transición Ecológica (MAATE), 2020).

This report provides a future simulation based on dynamic downscaling for the period 1985–2070. It models the likely temperature and precipitation conditions under two climate scenarios known as Representative Concentration Pathways (RCP 4.5 and RCP 8.5). The RCP 4.5 scenario suggests a probable temperature increase of at least 2 °C, while RCP 8.5 projects an increase greater than 3 °C (Armenta et al., 2016).

MAATE provided daily temperature and precipitation data in cells or pixels with defined central points. The pixels used were: F17C50, F17C51, F17C52, F17C53, F18C50, F18C51, F18C52, F18C53, F19C50, F19C51, F19C52, F19C53, each covering an area of 10,000 hectares. Additionally, discrepancies between *in situ* data and comparative data were assessed using percentage bias (BIAS) and root mean square error (RMSE) between the available historical data and the simulated data for each climate scenario over the 2011–2015 period (Equation 1 and Equation 2).

$$BIAS = \frac{\sum_{i=1}^{N}(P_i - O_i)^2}{N} \qquad (1)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(P_i - O_i)^2}{N}} \qquad (2)$$

Where P represents the predicted data, O the observed data, and N the total number of data points.

BIAS indicates the average tendency of the simulated data to be higher or lower than the observed data (Gupta et al., 1999). This statistic reaches its optimal value when it equals zero, which would indicate that the simulation is accurate (Moriasi et al., 2007). RMSE provides a measure of the average magnitude of forecast errors, which is equivalent to representing the standard deviation of the model errors (Righetti et al., 2019). The model's predictive capability improves as the RMSE value decreases, as this would indicate that the predictions are closer to the actual values (Arias-Muñoz et al., 2023).

The results obtained from the BIAS and RMSE analyses were used to determine whether correction of the climate data simulated by Ministerio de Ambiente, Agua y Transición Ecológica (MAATE) (2020) was necessary. Subsequently, using the validated climate data for the period 2025–2035, suitable areas for sugarcane cultivation were identified under the two climate scenarios: RCP 4.5 and RCP 8.5. This process included interpolation of annual temperature and annual precipitation for the 2025–2035 period using the Spline method in ArcGIS 10.8.2 (Figure 3). Finally, the simulated optimal zones were compared with the current optimal zones.

# 3 Results and Discussion

## 3.1 Current situation (Year 2022) and future projection of sugarcane (*Saccharum officinarum*) cultivation for the year 2031

Although sugarcane cultivation in the Chota Valley is not agroclimatically viable due to its failure to meet the minimum required precipitation of 1,200 mm, supervised classification of land cover and land use indicates that sugarcane is nonetheless cultivated in the area. In fact, between 1999 and 2011, a decrease of 1.4% in the cultivated area was observed.

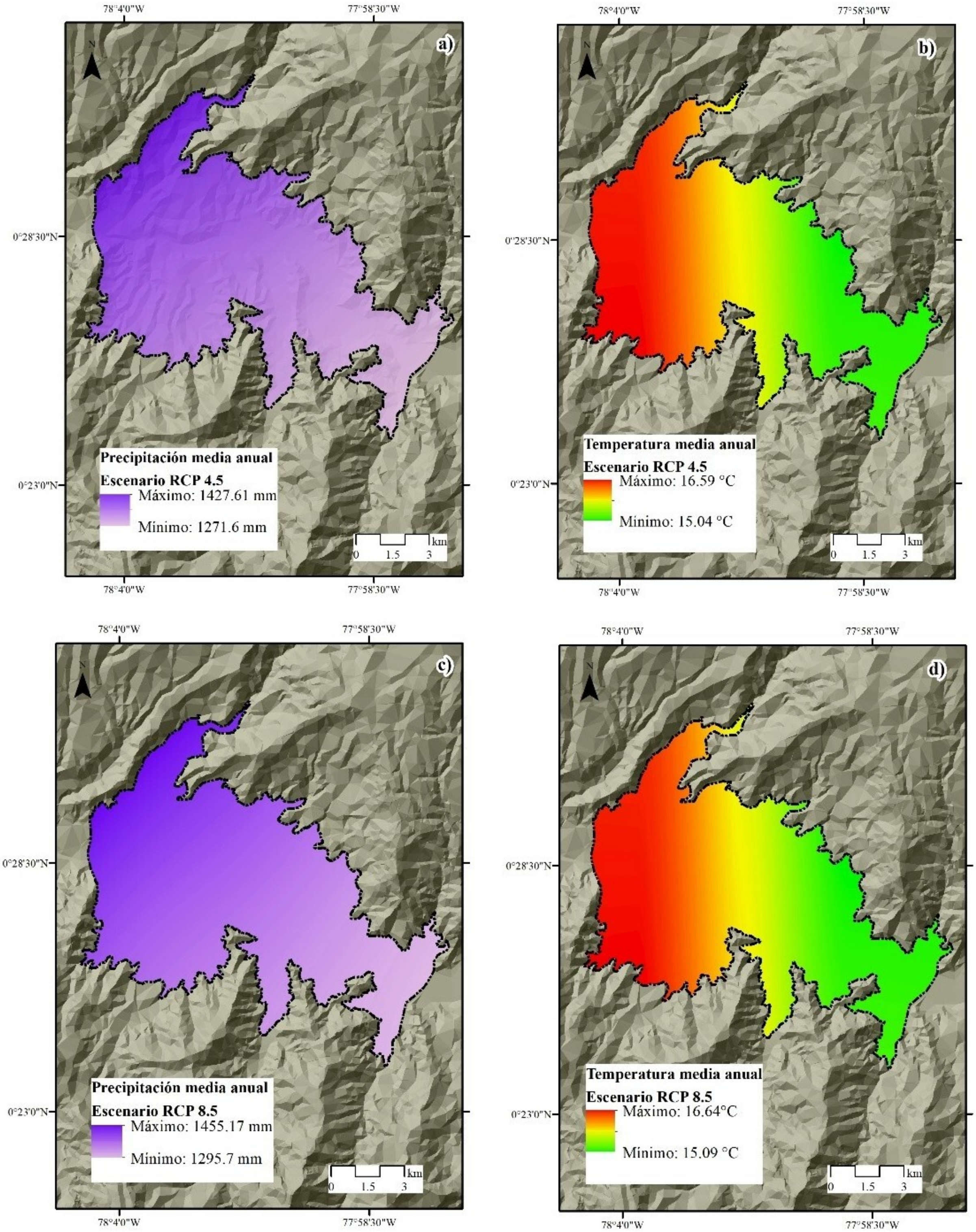


**Figure 3.** Interpolation of future precipitation and temperature for the period 2025-2035. a) Precipitation under RCP 4.5 scenario, b) Temperature under RCP 4.5 scenario, c) Precipitation under RCP 4.5 scenario, e) Temperature under RCP 8.5 scenario.

By 2011, sugarcane covered 530.52 hectares, representing 5.7 % of the total area. Thus, despite the absence of favorable climatic conditions, the crop is consistently cultivated in this territory. This persistence may be attributed to the use of two irrigation methods-drip and gravity irrigation-as well as the inherent adaptability of the species. By 2022, sugarcane cultivation occupied 481.52 hectares, equivalent to 5.21 % of total land cover, representing a 0.49 % decrease compared to 2011. Currently, sugarcane in the Chota Valley is cultivated by local farmers, and the harvest is generally sold to the Ingenio Azucarero del Norte (IANCEM). In contrast to sugarcane, other crops and urban settlements have shown greater expansion, increasing by 6.91 % and 2.66 %, respectively.

Based on simulated data for the near future, further changes are anticipated. By the year 2031, the sugarcane cultivation area is projected to decline from 481.52 hectares in 2022 to 410.99 hectares, representing a reduction of 14.65 %. This suggests that sugarcane will continue to be replaced by other crops such as mango, common beans, and chili peppers. As a result, these and other crops are expected to expand from 639.38 hectares to 681.36 hectares (an increase of 6.57 %), occupying approximately 7.37 % of the total area of the valley. Additionally, urban settlement areas are projected to grow by approximately 10.46 %, from 245.80 hectares to 271.52 hectares, primarily replacing semi-arid zones in the territory (Figure 4).

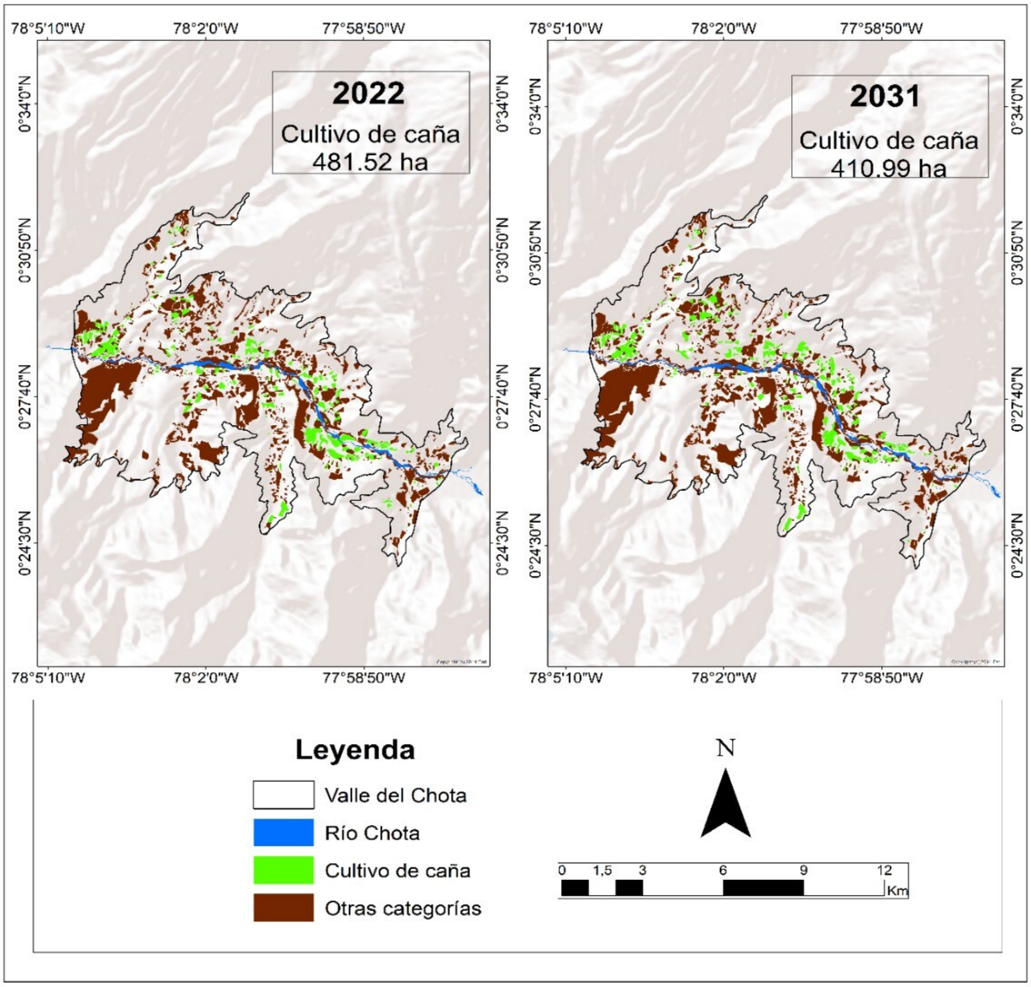


**Figure 4.** Land cover and land use change model for the period 2022–2031

## 3.2 Zoning of Sugarcane Cultivation under Climate Change Scenarios for the Period 2025–2035

First, it was determined that there is no need to correct the climate data simulated by Ministerio de Ambiente, Agua y Transición Ecológica (MAATE) (2020), as in both climate scenarios (RCP 4.5 and RCP 8.5), the values obtained for percentage bias (BIAS) are close to zero, and the RMSE values for precipitation fluctuate at most up to 2 mm, while those for temperature are close to zero (Table 6). These relatively low RMSE values suggest that the predictions are close to the actual values.

Consequently, for the 2025–2035 period, changes in precipitation and temperature are projected to affect sugarcane production in the Chota Valley as a result of climate change. Under both the RCP 8.5 and RCP 4.5 scenarios, the areas suitable for sugarcane cultivation are expected to increase proportionally, with optimal zones rising from currently nonexistent to covering 70.83 % of the area in both climate scenarios (Table 7 and Figure 5). This is because the average annual precipitation under these scenarios is projected to range between 1,261 mm and 1,455 mm, and the average annual temperature is expected to vary between 15 °C and 16.65 °C (Figure 3), thereby meeting the climatic requirements for the optimal development of the crop (Table 5). In this regard, according to the scenarios analyzed climate change will result in the emergence of optimal zones for sugarcane cultivation, in contrast to the current situation. As a result, it would be possible to cultivate sugarcane without relying on irrigation water supply.

Like other crops, sugarcane is not only influenced by climatic conditions during the agricultural year but also by the level of interest that farmers have in cultivating it (Silva et al., 2014). The reduction in sugarcane cultivation area from 1999 to 2022 demonstrates a gradual decline in the interest of local farmers in this crop. This trend persists despite the presence of the Ingenio Azucarero del Norte (IANCEM), a sugar mill located in the area that sustains product demand-a condition that, according to Moreno Izquierdo et al. (2018), supports optimal yields in regions with suitable natural conditions for sugarcane production. However, due to fluctuating prices and the crop's 18-month vegetative and production cycle, farmers reported in interviews that they prefer crops with shorter production cycles and greater market demand in nearby cities.

Espín Díaz (1999) noted that farmers in the valley have historically favored other crops over sugarcane due to easier market access. As a result, sugarcane is being replaced by crops such as mango (*Mangifera indica*), cucumber (*Cucumis sativus*), chili pepper (*Capsicum annuum*), and common bean (*Phaseolus vulgaris*). These crops not only have shorter growing cycles but also respond positively to the climatic and edaphic conditions of the region. Consequently, the area dedicated to sugarcane cultivation is not expected to increase by 2031, and the trend toward substitution with other crops will likely continue. The primary reasons are the declining interest and the need for faster-return crops. In general, studies conducted in Ecuador have shown that cultivated areas are projected to increase in various regions, such as the Amazonian forests (Heredia-R et al., 2021), the Chambo River basin in south-central Ecuador (Ross et al., 2017), and the Guayllabamba River basin (Abad-Auquilla, 2020). However, these studies do not specify which crops will replace others.

**Table 6.** BIAS and RMSE values between historical and simulated data for the climate variables precipitation and temperature

| Variable | Climate scenario | BIAS | RMSE |
|---|---|---|---|
| **Precipitation** | RCP 4.5 | -0.10 | 2.67 |
| | RCP 8.5 | -0.25 | 2.75 |
| **Average temperature** | RCP 4.5 | -0.41 | 0.64 |
| | RCP 8.5 | -0.45 | 0.66 |

On the other hand, the climatic influence on crop production highlights that climate variability in the region will increase the optimal zones for sugarcane cultivation in the Chota Valley. Under both RCP 4.5 and RCP 8.5 scenarios, precipitation is expected to increase between 1,271.6 mm and 1,427.61 mm in the former, and between 1,295.7 mm and 1,455.17 mm in the latter. According to Aguilar-Rivera et al. (2015), sugarcane requires at least 1,364.23 mm of water annually. Thus, climate change over the 2025–2035 period would meet the crop's water requirements. In some regions of Brazil, climate change has also been shown to enhance sugarcane production due to increased water availability (Marin et al., 2013). Similarly, Pereira De Souza et al. (2008) demonstrated that in controlled environments, higher $CO_2$ concentrations reduce stomatal conductance in sugarcane, thereby increasing photosynthesis. However, such physiological mechanisms could not be demonstrated in this study, nor were interactions between $CO_2$ and other climatic factors in natural conditions explored.

**Table 7.** Optimal areas for sugarcane cultivation in the Chota Valley under climate change scenarios for the period 2025-2035

| Period | Scenario | Optimum zone | | Suboptimal area | |
|---|---|---|---|---|---|
| | | Ha | % | Ha | % |
| 2025-2035 | RCP 4.5 | 6526.27 | 70.83 | 2687.91 | 29.18 |
| 2025-2035 | RCP 8.5 | 6526.27 | 70.83 | 2687.91 | 29.18 |

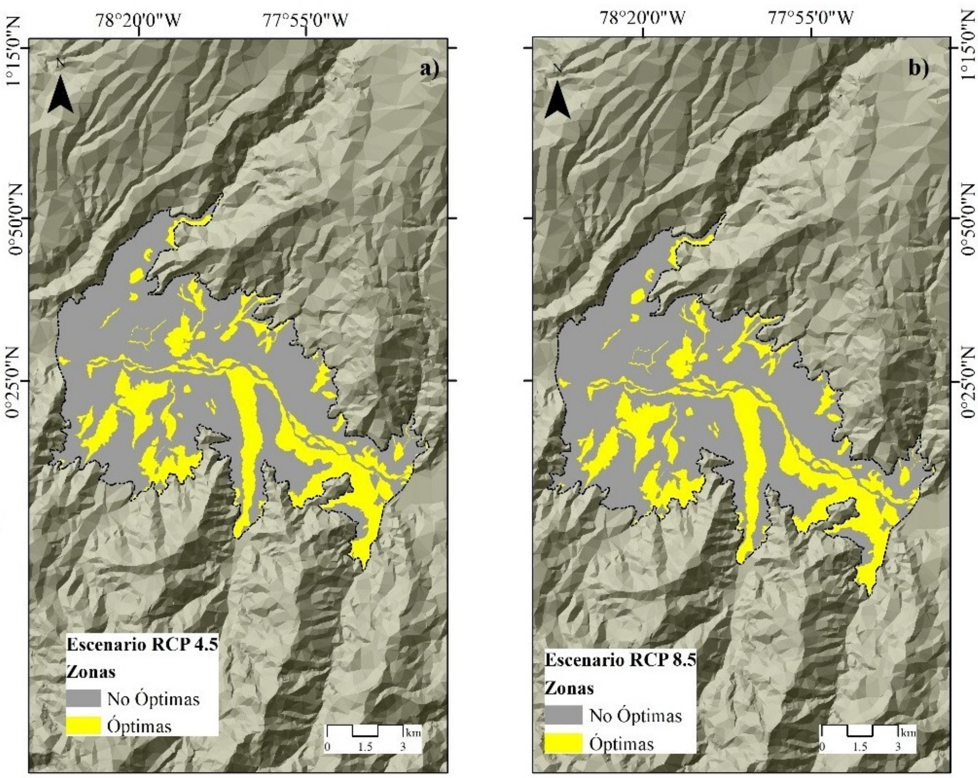


**Figure 5.** Zoning of sugarcane cultivation under climate change scenarios: a) Period 2025–2035, RCP 4.5 scenario; b) Period 2025–2035, RCP 8.5 scenario.

The negative impact on sugarcane yields is mainly associated with rising temperatures. Nevertheless, the projected temperature increase in the Chota Valley due to climate change is expected to remain between 15.04 °C and 16.64 °C, not exceeding the local minimum and maximum ranges of 13.2 °C and 26.7 °C. Therefore, no increase in water deficit is anticipated. This contrasts with the Southern Caribbean, where climate change is expected to cause a water deficit and result in yield reductions of 20% to 40% (Singh and El Maayar, 1998). Likewise, in Swaziland, sugarcane yields are expected to decline unless future models incorporate irrigation optimization (Knox et al., 2010). However, extreme weather events such as frosts may have less impact on crops like sugarcane, potentially allowing for increased yields (Todd et al., 2015).

Additionally, due to the inter-Andean location of the Chota Valley, other impacts of climate change may not significantly affect sugarcane production-unlike in Australia, for instance, where cultivated sugarcane areas are expected to expand further south of the tropics (Linnenluecke et al., 2020). Nonetheless, sugarcane production remains vulnerable to climate change, which increases the frequency and intensity of extreme weather events such as droughts, heatwaves, floods, and frosts (Jaiphong et al., 2016; Todd et al., 2015).

Therefore, the potential rise in such events in the Chota Valley could also negatively impact sugarcane yields. This is particularly true for drought, considering the region's warm climate, as climate change exacerbates water stress, affecting the plant's growth and development (Zhao and Li, 2015). Additionally, other constraints could influence the impact of climate change in the Chota Valley, such as the depletion of soil organic matter due to continuous cultivation, which, according to Aguilar-Rivera et al. (2015), increases soil vulnerability to climatic and environmental influences.

## 4 Conclusions

Despite the lack of optimal agroclimatic conditions, sugarcane cultivation has persisted for years in the Chota Valley. However, from 1999 to 2022, the cultivated area has steadily declined. This reduction, though not exceeding 2%, has led to sugarcane occupying 5.21% of the valley's total area as of 2022. Projected land use change is expected to cause a further reduction in sugarcane area by 2031, amounting to a 14.65% decrease compared to 2022. The primary reason for this decline is the replacement of sugarcane with other short-cycle crops. Most of the sugarcane is produced by local farmers, and although the Ingenio Azucarero del Norte purchases their product, growers perceive the crop's long growth cycle as economically unsustainable. Therefore, they opt for crops with shorter harvest times, such as beans and chili peppers.

The effects of climate change under both RCP 4.5 and RCP 8.5 scenarios are projected to be beneficial for sugarcane cultivation, as the future period (2025–2035) will see an increase in optimal growing areas by approximately 70.83% compared to the current situation. This is due to increased precipitation in both scenarios, surpassing the crop's minimum water requirement of 1,364.23 mm. Moreover, the negative impact of temperature is expected to be minimal, as temperatures will remain within the historical range, not exceeding 16 °C to 19 °C.

Ultimately, the findings of this study demonstrate that maintaining optimal growing zones alone is insufficient to sustain interest in sugarcane production. The potential positive effects of climate change in the Chota Valley, such as improved water availability, may not be enough to prevent the replacement of sugarcane with other crops that generate greater interest among farmers, whether due to shorter production cycles or higher profitability.

## Authors' contribution

P.A.M: Conceptualization, data curation, formal analysis, methodology, project administration, resources, supervision, validation, visualization, original draft structure, writing-review and editing. E.L.Ch.B.: Data curation, research, methodology, original draft writing. S.A.P.Y.: Data curation, research, methodology, original draft writing. G.J.A.: Validation, visualization, supervision, writing-review and editing. O.R.: Validation, visualization, supervision, writing-review and editing.

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Cellular and Molecular Biology

# POTENTIAL EFFECTIVENESS OF VITRAKVI (LAROTRECTINIB) FOR TREATING TYPES OF SOLID TUMORS CARRYING NTRK GENES AND THE IMPACT OF TRKC MUTATIONS

## Eficacia de *vitrakvi* (larotrectinib) para el tratamiento de tumores sólidos portadores de genes *ntrk* y el impacto de las mutaciones *TRKC*

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**Abstract**

Vitrakvi is a cancer treatment that targets solid tumors with NTRK gene fusion. NTRKs are rare genetic effects that can arise in tumors from several organs, including the lungs, thyroid glands, and intestines. This study sought to identify the position at which Vitrakvi binds to tropomyosin receptor kinases (TRKs) as well as the effects of TRKC mutations on the fusion site. Materials and PubChem were used to obtain the chemical structure of Vitrakvi. The 3D structure of TRKs was derived from the PDB. Docking was implemented via AutoDock Vina. Docking, visualization, and sequence reconstruction were completed via the PyMol, BIOVIA, and PyRx programs. The fusion of Vitrakvi with TRKA and TRKB is altered if they are combined with their respective stimulators (BDGF and NT-4/5). TRKC combines with Vitrakvi in the same chain in which it is coupled to its stimulator (NT-3), but the fusion site shifts away from the triple mutation site. Even though clinical trials of TRK inhibitors have just started, there is reason to be hopeful for people with TRK mutations and the field of molecularly targeted medicines.

***Keywords***: Vitrakvi, TRK, Solid cancer, NT-3, Mutation, Docking.

**Resumen**

Vitrakvi es un tratamiento oncológico dirigido a tumores sólidos que presentan fusiones génicas del gen NTRK. Las alteraciones en los genes NTRK constituyen efectos genéticos poco comunes que pueden manifestarse en tumores originados en diversos órganos, incluidos los pulmones, las glándulas tiroideas y el intestino. El objetivo de este estudio fue identificar el sitio de unión de Vitrakvi a las quinasas del receptor de tropomiosina (TRK), así como evaluar los efectos de las mutaciones en TRKC sobre el sitio de fusión. Para obtener la estructura química de Vitrakvi se utilizaron recursos como PubChem, y la estructura tridimensional de las TRK se obtuvo del Protein Data Bank (PDB). El acoplamiento molecular (docking) se llevó a cabo mediante el programa AutoDock Vina. Las simulaciones de acoplamiento, visualización y reconstrucción de las secuencias se realizaron utilizando los programas PyMol, BIOVIA y PyRx. La fusión de Vitrakvi con TRKA y TRKB se ve modificada cuando estas quinasas se combinan con sus respectivos estimuladores (BDGF y NT-4/5). En el caso de TRKC, la unión con Vitrakvi ocurre en la misma cadena que se acopla a su estimulador (NT-3); sin embargo, el sitio de fusión se desplaza respecto al sitio de la triple mutación. Aunque los ensayos clínicos con inhibidores de TRK se encuentran en fases iniciales, existen fundamentos prometedores para la esperanza tanto en pacientes con mutaciones en TRK como en el campo de las terapias dirigidas molecularmente.

***Palabras clave***: Vitrakvi, TRK, Cáncer sólido, NT-3, Mutación, Docking.

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# 1 Introduction

Vitrakvi, also known as larotrectinib, is a tyrosine kinase inhibitor that can be used to treat adults and children who have solid tumors that have one of the following characteristics: a neurotrophic receptor tyrosine kinase (*NTRK*) gene fusion without acquired surgical resection that is likely to result in severe morbidity, metastatic resistance mutation, no satisfactory alternative therapies, or post treatment progression. The FDA authorized Vitrakvi on November 26, 2018. The total response rate and length of response data were used as the primary justification for the expedited approval of larotrectinib ($C_{21}H_{22}F_6NO_2$) (Loxo Oncology, 2018; US Food and Drug Administration, 2018).

Because carcinomas of tropomyosin receptor kinase (TRK) fusion are uncommon, there is no homeostasis in tumor tissue where standard therapies are unavailable or recommended therapies exist but fail to provide documented and relevant clinical benefit, and patient transfer is possible; randomized controlled trials to demonstrate improvement are neither feasible nor appropriate(Wyatt et al., 1999).
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There are many different types of primary tumors, each with its own unique natural history, making it impossible to conduct a single randomized study on all of them. However, the data from the Vitrakvi trials were pooled to provide evidence of the efficacy and safety of each regulatory request (Amatu et al., 2016; Lange and Lo, 2018).

The overall response rate has been demonstrated to be a positive indicator of the efficacy of Vitrakvi in treating select groups of tumors. Quantitative differences in effects are possible on the basis of cancer type and additional genetic changes (Burris et al., 2015).

Vitrakvi is unlike many other cancer drugs since it is designed to attack tumors with a specific gene arrangement regardless of where they may be located in the body. Preliminary data demonstrate that it successfully reduces tumor size in patients. In addition, the rapidity with which tumors can be reduced is crucial for providing symptom relief to patients (Doebele et al., 2015; Laetsch et al., 2018).

Vitrakvi appears to be safe, and any potential harm from it seems to be minimal. Therefore, the European Medicines Agency decided that the benefits outweigh the dangers and that it has approved for use in the European Union (Figure 1).

Vitrakvi pills or liquid (20 mg/ml) can be used orally (25 and 100 mg). If the malignancy is stable and the side effects are acceptable, adults should take 100 mg twice a day. Weight determines the child's dose (US Food and Drug Administration, 2018).

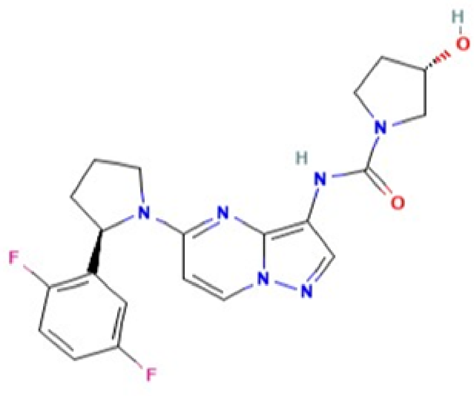

**Figure 1.** Chemical structure of Larotrectinib (Vitrakvi).

The primary objective of this study is to evaluate the molecular interactions between Vitrakvi (larotrectinib) and TRKs (TRKA, TRKB, and TRKC), particularly focusing on the impact of TRKC mutations on drug binding and efficacy.

## 1.1 Mechanism of action

TRKs are proteins that are found in human neural tissue. They become active as a result of the action of neurotrophins, which allows them to play a significant role in the physiological processes that underlie the development of the nervous system as well as its functions. The optimal levels of neuronal development, differentiation, and survival are regulated by the TRKs TRKA, TRKB, and TRKC when they engage in a dynamic interaction with neurotrophic autoligands (Yang et al., 2022). The genes NTRK1, NTRK2, and NTRK3 encode TRKA, TRKB, and TRKC, respectively.

Fusion proteins have been identified as a result of chromosomal rearrangements such as in-frame fusion of these genes with a variety of partners, translocations in the TRK domains, mutations in the TRK binding area, NTRK amplification, and the production of TRK splice variants. Activated TRK chimeras that can act as tumor inducers enhance cancer cell line proliferation and survival (Ardini et al., 2016; Tacconelli et al., 2004).

Gene fusions result in the synthesis of chimeric TRK proteins, which either have inherent kinase activity or overexpress the kinase domain. This changed state, which can be induced by point mutations, chromosomal rearrangements, gene fusions, or deletions, results in spontaneous ligand-independent dimerization, which activates the signal transduction pathway (Arevalo et al., 2000). To date, it has been established that all of the known mechanisms for oncogenic TRKA activation involve truncation of the extracellular domain (Bové et al., 2021).

According to one study, the expression of the gene encoding a neurotrophin's cognate TRK receptor is regulated by the physiological levels of the neurotrophin in vivo, although this mechanism of regulation only occurs in a portion of the cells that express the receptor (Raedler, 2019).

Vitrakvi has shown anticancer effects in *in vitro* and *in vivo* tumor models, with constitutive activation of TRK proteins caused by gene fusion, protein regulatory domain loss, or overexpression of the TRK protein. Vitrakvi has demonstrated little efficacy in cell lines harboring TRKA kinase domain point mutations, including the clinically characterized acquired resistance mutation. F617L, G623R, and G696A are point mutations in the TRKC domain associated with clinically recognized acquired resistance to Vitrakvi (Vaishnavi et al., 2013; Hashimoto et al., 2005).

Like other receptor tyrosine kinases, TRK proteins are activated when a ligand binds to the receptor's extracellular domain. Initially identified and classified as proteins that promote sympathetic and sensory neuronal growth and survival, neurotrophins are proteins that are secreted and act as ligands for TRK proteins (Coppola et al., 2004).

For every kinase, the individual ligands are denoted by the acronym NGF, which stands for nerve growth factor. The brain-derived growth factor (BDGF) is for the TRKA receptor, whereas NT-4/5 and NT-3 are, respectively, for the TRKB and TRKC receptors. There is a ligand-binding region, a transmembrane region, and an intracellular domain that includes a kinase domain. It regulates the latter stages of cell division, axon and dendrite growth, and branching. They also perform more nuanced roles that have nothing to do with the neurological system (Ferrer et al., 1999; Dwivedi et al., 2003).

NGF stimulation of TRKA has been connected to the modulation of pain, itch, and inflammation in addition to its function in the growth and maintenance of cholinergic, sympathetic, and sensory neurons (Ernst et al., 2009). TRKB is triggered by BDNF and has been proven to improve neuronal plasticity and survival. TRKB inhibition as a therapeutic target may result in unpleasant side effects such as ataxia, lethargy, anhedonia, and depression (Ivanov et al., 2013).

NGF binding to the TRKA receptor activates the Ras/MAPK pathway, resulting in increased proliferation and cellular growth via extracellular kinase (ERK) signaling. Other pathways, such as phospholipase C (PLC) and phosphatidylinositol-3 kinase (PI3K), are also active (Nakagawara, 2001; Boulle et al., 2012).

Neurotrophin-3 (NT-3) is a growth factor that affects some nerve cells. It promotes the creation and differentiation of new neurons and synapses, as well as the survival of existing neurons (Chaldakov et al., 2004). NT-3 stimulates cell migration via TRKC. Inhibitors such as K252a and Vitrakvi prevent this effect from occurring. Hirschsprung's disease is characterized by gastrointestinal issues and the absence of neurons in the muscular plexus and submucosa. TRKC mutations that result in an inactive protein have been identified as a cause of Hirschsprung's disease, which is defined by these characteristics (Keeler et al., 2017).

Some malignancies with TRKA protein expression may have a better prognosis than others do. In the case of neuroblastoma, for example, TRKA expression is associated with a favorable prognosis because TRKA and NGF signaling may play a tumor suppressor role by inducing differentiation, growth arrest, and angiogenesis in neuroblastoma cells. The expression and signaling of TRKC are related to a neuroblastoma phenotype that is more aggressive and invasive, whereas higher TRKA expression is associated with favorable clinical characteristics. The clinical characteristics of TRKC and TRKB are more aggressive, indicating that this hopeful outlook may apply exclusively to TRKA.

TRKB promotes angiogenesis and resistance to anticancer treatments by promoting autocrine and paracrine signaling in cancer cells. Despite the fact that this feature has been observed only in neuroblastoma, it is likely that it applies to other types of cancer as well. Furthermore, NTRK1 rearrangement in papillary thyroid carcinomas is associated with a poorer prognosis than that in patients without this fusion gene. On the other hand, the invention and improvement of kinase inhibitor medicines, targeted tumor methods, and scarcity of clinical resistance mechanisms may make NTRK genes that affect malignancies more treatable (Lange and Lo, 2018; Vaishnavi et al., 2015).

## 2 Materials and methods

The chemical structure of Vitrakvi was retrieved from PubChem and molecular docking was conducted using AutoDock Vina. The three-dimensional structures of TRKA, TRKB, and TRKC were obtained from the PDB database, and docking visualizations were performed using PyMol, PyRx, and BIOVIA software. Mutagenic models of TRKC (F617L, G623R, and G696A mutations) were constructed to analyze the effects of these mutations on binding efficacy. The chemical structure of Vitrakvi was obtained from PubChem via the identifier CID (46188928). TRKs with the following PDB IDs were selected: TRKA (4F0I), TRKB (4ASZ), and TRKC (6KZD). The most essential aspect is that these receptor kinases can be activated by combining them with other ligands. We used Vitrakvi for the molecular docking of these combination kinases.

Brain-derived growth factor (BDGF) is coupled with TRKA under PDB ID (1WWW). TRKB is coupled with neurotrophin-4/5 under ID (1HCF). Neurotrophin-3 (NT3), the NKRC receptor, was acquired from PDB under the ID (1B8K). Using AutoDock Vina, molecular docking between Vitrakvi and TRKs was performed. To accomplish docking tasks, we used the BIOVIA Discovery Studio 2021 Visualizer and the Python package PyRx. Each molecule docking was viewed in three dimensions with PyMol. The 3-point mutations in TRKC were built via the PyMol molecular modeling program.

## 3 Results

Molecular docking results demonstrated high binding affinity between Vitrakvi and wild-type TRKA, TRKB, and TRKC, with binding energies of -9.4, -8.8, and -9.9 kcal/mol, respectively. However, TRKC mutants exhibited significant conformational shifts at the binding site, specifically in the F617L mutant, indicating a potential mechanism of drug resistance. Furthermore, when TRKs interacted with their respective ligands (BDGF, NT-4/5, and NT-3), the binding affinity of Vitrakvi decreased, highlighting competitive binding dynamics. TRKs consist of three subunits: TRKA, TRKB, and TRKC.

Molecular docking of proteins with Vitrakvi was subsequently conducted, and the proteins were subsequently assembled into the TRK structure. Figure 2 shows the constituents of the natural TRKs that interact with Vitrakvi. Table 1 displays the interaction energy, also known as the binding affinity, as well as the relative mean square deviation

(RMSD) of the atoms between the protein and ligand. When the RMSD between models was zero, it was chosen as the best interaction with high affinity.

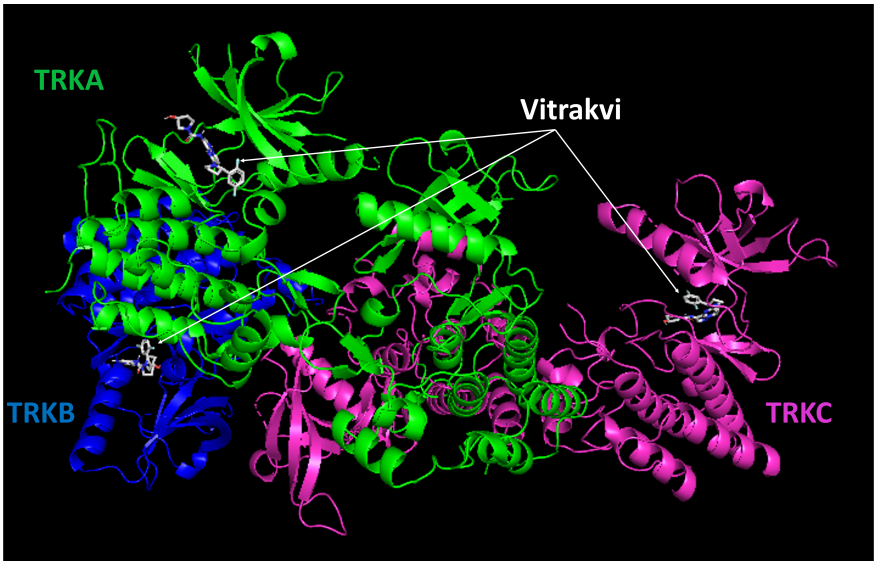


**Figure 2.** Molecular docking of Vitrakvi with normal TRKA (green), TRKB (blue) and TRKC (violet).

### 3.1 TRKA-Vitrakvi interaction poses:

The interaction poses between TRKA and Vitrakvi are illustrated in Figure 3. The types of bonds and residues used were as follows: conventional hydrogen bonds (ARG591 and SER671), carbon hydrogen bonds (MET591, ARG592 and GLY516), fluorine bonds (ARG653), alkyl and pi-alkyl bonds (LEU515), pi-sigma bonds (ILE674) and pi-sulfer bonds (MET670).

### 3.2 TRKB-Vitrakvi interaction poses:

The interaction poses included conventional hydrogen bonds (ASP710), carbon hydrogen bonds (GLU604 and GLY709), fluorine interactions (HIS690), alkyl and pi-alkyl interactions (LEU608, PHE633 and ILE616), and amide pi-stacking interactions (ILE708).

### 3.3 TRKC-Vitrakvi interaction poses:

The interaction poses included conventional hydrogen bonds (ASP624 and ARG683), carbon hydrogen bonds (LEU544 and MET620), alkyl and pi-alkyl interactions (ALA570 and LEU686), pi-sigma interactions (VAL552), and pi-pi T-shapes (PHE698).

The anticancer activity of Vitrakvi has been demonstrated in both *in vitro* and *in vivo* tumor models, namely, in cells whose TRK proteins are constitutively activated as a result of gene fusion or deletion of the protein's regulatory region. The point mutations G623R, G696A, and F617L in the TRKC domain confer resistance to Vitrakvi. The alterations were uploaded to the molecular fusion prototype between TRKC and Vitrakvi. A study of the fusion data revealed that the attachment region has a definite effect and that there is a considerable difference between the wild-type fusion model and the mutagenic model (Figure 4).

The second type of molecular docking occurs when kinases and Vitrakvi form complexes with NGFs. When TRKA combines with BDGF, it docks with Vitrakvi at two residues (HIS353 and VAL354). When TRKB interacts with NT-4/5, it forms a fusion with the ASN325, ILE330, MET354, and ASN355 residues. Various types of bonds are observable because of these interactions (Figure 5).

**Table 1.** The best docking models with high affinity and degree of RMSD.

| Interaction | Binding affinity | RMSD/Lower bond | RMSD/Higher bond |
|---|---|---|---|
| *TRKA-Vitrakvi* | *-9.4* | *0* | *0* |
| = | -9 | 37.952 | 40.5 |
| = | -8.9 | 31.582 | 34.651 |
| = | -8.9 | 38.86 | 40.942 |
| = | -8.8 | 2.959 | 4.916 |
| = | -8.8 | 20.467 | 23.779 |
| = | -8.8 | 3.334 | 6.68 |
| = | -8.7 | 39.125 | 41.494 |
| = | -8.6 | 34.193 | 38.61 |
| *TRKB-Vitrakvi* | *-8.8* | *0* | *0* |
| = | -8.3 | 3.949 | 5.795 |
| = | -8.2 | 11.7 | 14.007 |
| = | -8.2 | 3.016 | 3.988 |
| = | -8.2 | 3.818 | 6.713 |
| = | -8.1 | 13.205 | 15.731 |
| = | -8 | 15.184 | 17.403 |
| = | -7.9 | 2.873 | 4.3 |
| = | -7.5 | 14.745 | 17.109 |
| *TRKC-Vitrakvi* | *-9.9* | *0* | *0* |
| = | -9.8 | 60.896 | 62.482 |
| = | -9.7 | 61.139 | 62.664 |
| = | -9.4 | 59.204 | 61.71 |
| = | -9.4 | 3.145 | 5.358 |
| = | -9.4 | 4.198 | 6.607 |
| = | -9.3 | 2.309 | 3.632 |
| = | -9.3 | 3.498 | 6.198 |
| = | -9.2 | 4.166 | 7.191 |

Table 2 contains both the binding affinity and the RMSD of the atoms that are found between the ligand and the protein. The best interaction model with high electrostatic affinity energy when the RMSD is equal to zero.

Notably, the situation is different in the case of TRKC, which has the ability to fuse with a ligand (NT-3). For the first two types of kinases (TRKA and TRKB), the interaction between the ligand and the kinase was dependent on the chains that the Vitrakvi did not directly connect with. With respect to TRKC, the ligand and Vitrakvi are fused together in the same chain.

NT-3 (1B8K) is considered an inhibitor of TRKC's current activity in the presence of another drug; thus, the docking operation was performed on TRKC, NT-3, and Vitrakvi, as illustrated in Figure 6. The docking site of TRKC appears at the LYS49, TYR51, and ARG87 residues. The results of the triple fusion clearly revealed that the Vitrakvi fusion site shifted from its initial position. The binding affinities and RDDs are presented in Table 3.

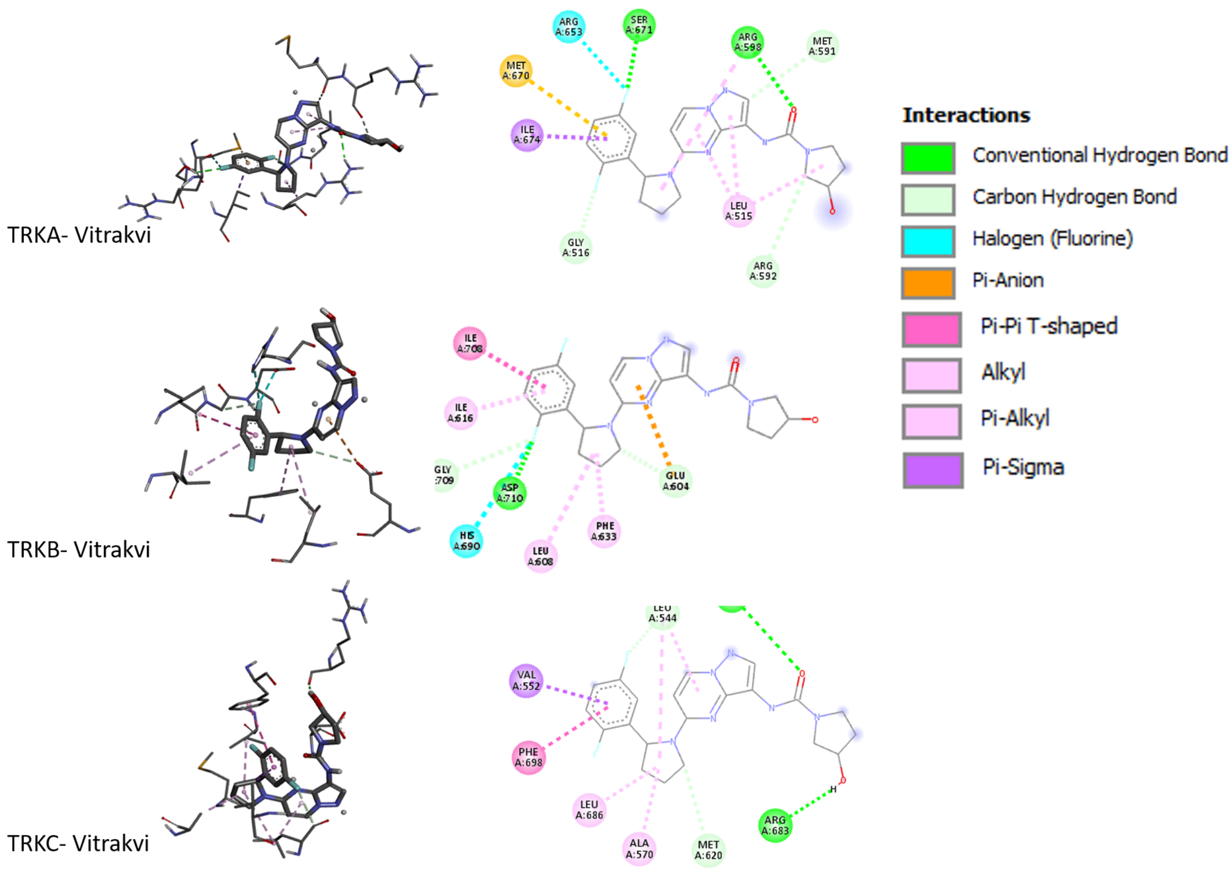


**Figure 3.** Pose interactions between TRKs and Vitrakvi with a variety of bonds.

# 4 Discussion

This study elucidates the intricate relationship between Vitrakvi's binding mechanisms and TRK mutations, providing critical insights into the role of molecular docking in predicting drug efficacy and resistance. The observed shifts in binding affinity and conformation for TRKC mutants underscore the importance of targeting specific mutations to enhance the therapeutic efficacy of Vitrakvi. The development of molecular diagnostic methods has led to the identification of an increasing number of neoplastic abnormalities, such as gene activation, point mutations, insertions, frame-shift deletions, and amplifications or rearrangements, which has had a significant impact on the treatment of solid tumors in recent years. A significant change in the way solid tumors are treated has resulted from the identification of an increasing number of neoplastic abnormalities, such as gene activation point mutations, insertions, in-frame deletions, and amplifications or rearrangements, with the development of molecular diagnostics (De Braud et al., 2014; Federman and McDermott, 2019).

By using these mutations as predictive biomarkers, precision medicine aims to provide tailored care. Molecular alterations resulting in constitutively active fusion proteins have recently come to the forefront as targets for cancer therapy. TRK proteins are examples of targets exploited in cancer treatment. In addition, methods of sequencing of the future generation are helpful for discovering gene fusions in an objective manner, which helps to increase research in this area (Hong et al., 2020).

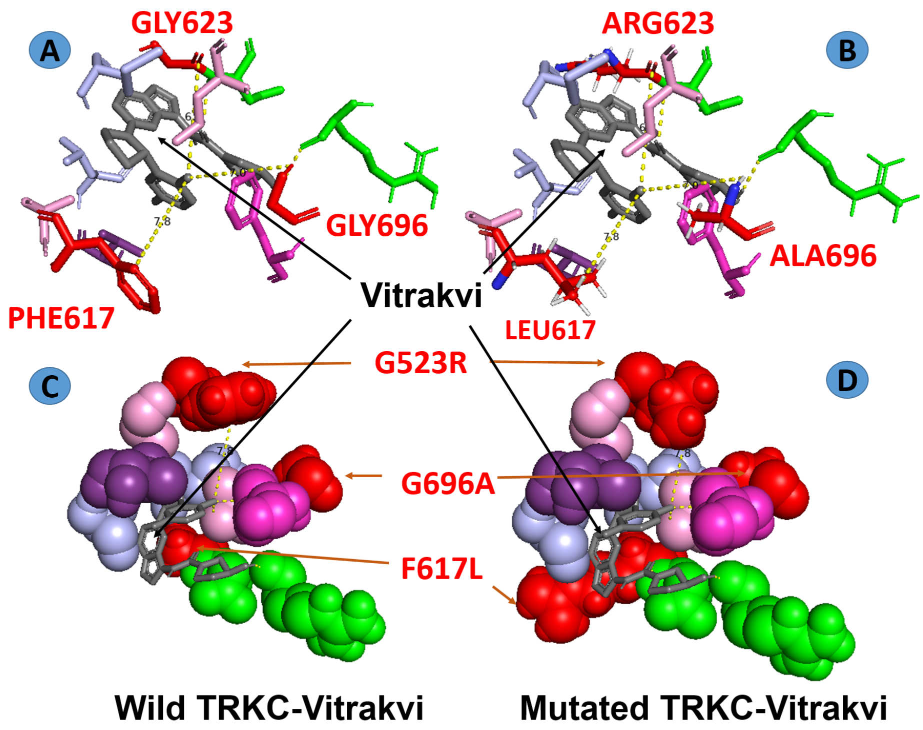


**Figure 4.** Molecular docking of TRKC-Vitrakvi in wild-type and mutated TRKC. A: Licorice wild docking image showing the interaction poses and residues (PHG617, G623 and G696). B: Licorice mutation docking image showing the mutated residues (LEU617, ARG623 and ALA696). C: Wild-type Spheres docking with the red residues of interest. D: Sphere mutation docking results in different red residue forms.

Tumorigenicity occurs when several distinct neoplastic tissues are brought together with a shared tumor driver. There is a growing need to normalize data from studies of novel medicines to data reported for historically accessible therapies across tumor tissues as more tumor-agnostic studies are conducted. There is currently no foolproof method for gauging the clinical efficacy of these treatments in individual tumor types. The data will likely show historical and within-patient comparisons across subsequent treatment lines, and several methods are anticipated to play crucial roles in understanding these data across tumor tissues (Laetsch and Hong, 2021; Cocco et al., 2019).

TRK fusion cancer is traditionally treated with a combination of chemotherapy, biologic therapy, or immunotherapy, which is based mostly on tumor histology. Vitrakvi is a drug that has been approved in the US and Japan as the first and only way to treat solid tumors in adults and children over 12 years of age that have an NTRK gene fusion (Megan et al., 2021). Vitrakvi was purposefully developed as an ATP-competitive and selective RTK inhibitor to prevent the activity of off-target kinases (Drilon et al., 2022).

In three clinical trials including 55 adults and children with solid tumors and an NTRK gene fusion without a resistance mutation, Vitrakvi proved effective. These patients had cancer that advanced after treatment or had no effective alternatives. Vitiligo responds to 75% of solid tumors. These reactions were persistent, with 73% lasting at least six months and 39% lasting a year or more at the time of the outcome analysis (Doz et al., 2022).

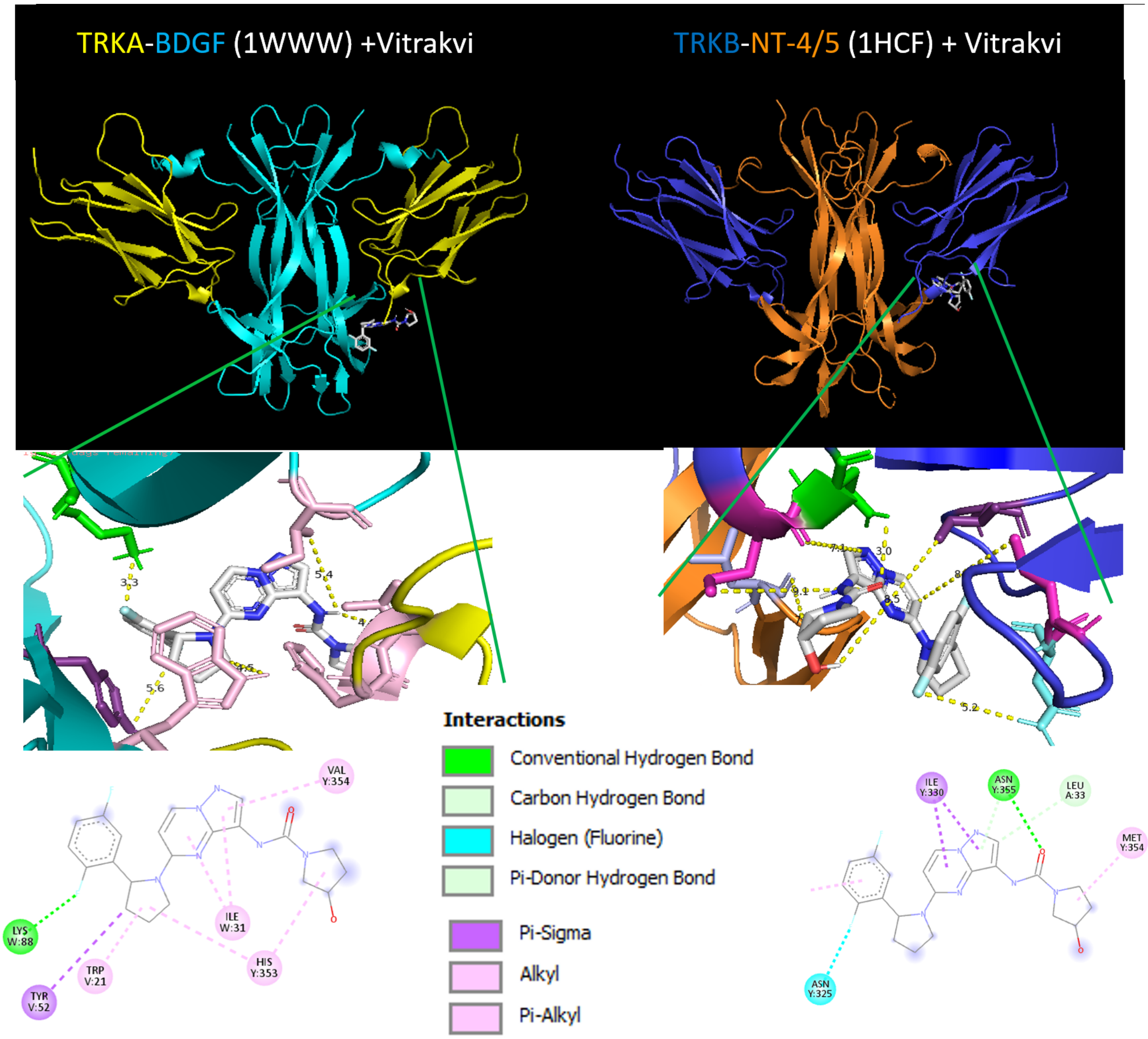


**Figure 5.** Molecular interactions between kinases and Vitrakvi when their favorite ligands are combined. Left: TRKA+BDGF (1 WWW). Vitrakvi is in contact with TRKA in HIS353 and VAL354, whereas it contacts BDGF in TRP21, ILE33, TYR52, and LYS88. Right: TRKB+NT-4/5 (1HCF). Vitrakvi is in contact with TRKB in ASN325, ILE330, MET354, and ASN355, whereas it contacts NT-4/5 in LEU33.

In the present study, complete fusion was carried out between the TRK proteins. Vitrakvi was docked to each component of kinases (TRKA, TRKB, and TRKC). When the RMSD was equal to zero, the optimal model with high binding affinities for each kinase was determined: -9.4, -8.8, and -9.9, respectively. Fusions have revealed several forms of bonds, with typical hydrogen bonds occurring most commonly.

Viktrakvi is an effective anticancer drug that acts by either blocking the activity of fusion kinases or deleting the regulator protein domain in cells that overexpress kinases. Both of these mechanisms work together to combat cancer. However, research has shown that specific point mutations in TRKC (F617L, G623R, and G696A) can confer resistance to Vitrakvi (Vaishnavi et al., 2013). The wild-type TRKC and Vitrakvi successfully docked with each other.

The three-dimensional structure of the mutated TRKC carrying the triple mutation was subsequently reconstructed. As a consequence of the action of the medication, differences in the configuration of the anchoring site were found between the wild-type kinase and the mutant kinase that was being evaluated. The most significant alteration in the shape of the fusion was observed in the F617L mutant in the sphere view, where the residues underwent a distinct conformational change that influenced the result of the fusion.

The kinases can also act with other growth factors; hence, we docked the receptor to the activator in addition to Vitrakvi. To stimulate, it is well known that TRKA is paired with BDGF and that TRKB is combined with NT-4/5. Complexes of (1WWW) and (1HCF) were selected for docking with Vitrakvi. For each docking, a distinct set of bonds is established. Once TRKA and TRKB were docked with Vitrakvi, the binding affinity scores were recorded to a lesser degree than before. The binding energy was -8.9 for TRKA-BDGF and -7.4 for TRKB-NT-4/5. These data showed for the first time that docking Vitrakvi with TRKA or TRKB is superior to docking with TRKA-BDGF or TRKB-NT-4/5.

In the present work, the binding of Vitrakvi to TRKA or TRKB alone differed from the docking site when both kinases were coupled with BDGF or NT-4/5, respectively. The problem is slightly different for TRKC because the location where the mutations take place is in the same region of fusion with Vitrakvi. In contrast to several other types of kinases (TRKA and TRKB), the TKRC activator, also known as NT-3, combines with the same chain. Figure 5 shows that in the association of Vitrakvi with TRKC, the location of the intercalation fusion changed in the direction of neurotrophin-3, away from the location where the mutation took place. This helps to explain why the mechanism changed the direction of the intercalation from its original docking area in the event that there were triple mutations. In addition, the binding energy between TRKC alone and Vitrakvi was greater when TRKC was paired with NT-3.

**Table 2.** The best docking models with high affinity and degree of RMSD between 1WWW and 1HCF with Vitrakvi.

| Interaction | Binding affinity | RMSD/Lower bond | RMSD/Higher bond |
|---|---|---|---|
| *1WWW- Vitrakvi* | *-8.9* | *0* | *0* |
| = | -8.8 | 5.023 | 3.039 |
| = | -8.4 | 16.653 | 14.031 |
| = | -8.3 | 18.73 | 16.162 |
| = | -8.2 | 3.112 | 1.677 |
| = | -8 | 17.109 | 14.566 |
| = | -7.7 | 16.675 | 13.961 |
| = | -7.7 | 16.51 | 13.609 |
| = | -7.7 | 7.245 | 4.608 |
| *1HCF- Vitrakvi* | *-7.4* | *0* | *0* |
| = | -7.3 | 14.887 | 12.102 |
| = | -7.3 | 37.06 | 34.562 |
| = | -7.1 | 2.325 | 1.746 |
| = | -7.1 | 29.87 | 26.509 |
| = | -7 | 36.186 | 34.368 |
| = | -7 | 15.02 | 12.533 |
| = | -7 | 22.616 | 19.816 |
| = | -6.9 | 36.138 | 32.961 |

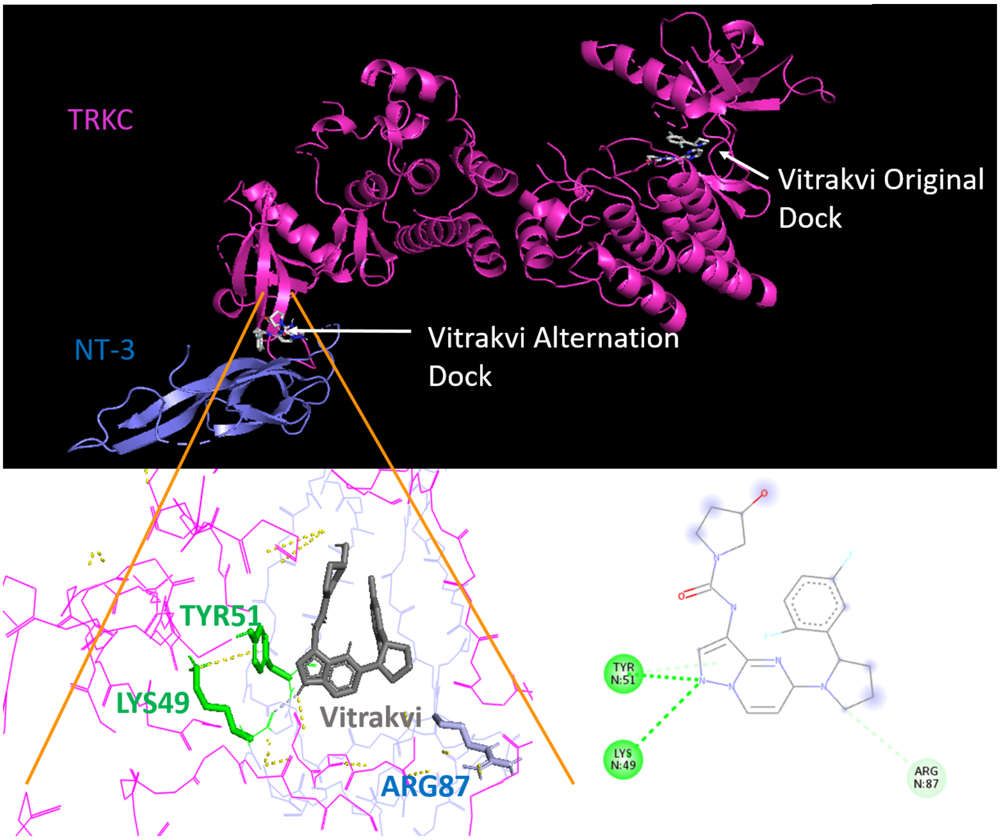


**Figure 6.** Changes in the molecular interaction of the original position docking between TRKC and Vitrakvi on the right. New fusion of Vitrakvi with TRKC when a kinase is combined with NT-3 (1B8K) is shown on the left. Two conventional hydrogen bonds are present in the LYS49 and TYR51 residues of TRKC. Carbon–hydrogen bonds appear in the ARG87 residue of TRKC.

Both humans and rodents express Neurotrophin-3 as well as its corresponding receptor TRKC. The level of NT-3 expression decreases as people age. The expression of NT-3 is most commonly detected in blood vessels that are in charge of irrigating adipose tissue, whereas the expression of TRKC is equivalent in both isolated adipocytes and total tissue (Bové et al., 2021).

Virakvi is approved for individuals with solid tumors, and this biomarker is not a suitable alternative treatment for their progressing metastatic condition. Vitrakvi is an innovative step forward in both the field of precision medicine and the development of oncology drugs because it is a tissue-agnostic treatment that has been shown to be safe and to produce long-lasting effects in this patient population (Vaishnavi et al., 2013).

Since the overexpression of NTRK genes contributes to carcinogenesis and progression, TRK inhibitors may be beneficial for treating malignancies with abnormal NTRK signaling. Mutations in NTRK genes are found in a range of cancer tissues; hence, research and investment in TRK inhibitors may be novel and effective treatments for many cancers (Lange and Lo, 2018).

However, the development and improvement of kinase inhibitor therapies, in conjunction with the identification of an oncogenic target and the relatively restricted expression of clinical resistance mechanisms, may prove to be effective in making cancers caused by NTRK gene changes more manageable.

**Table 3.** The best docking models with high affinity and degree of RMSD between TRKC+ NT-3 and Vitrakvi.

| Interaction | Binding affinity | RMSD/Lower bond | RMSD/Higher bond |
|---|---|---|---|
| *(TRKC+NT-3)- Vitrakvi* | *-7.3* | *0* | *0* |
| = | -7 | 5.217 | 2.451 |
| = | -6.9 | 5.903 | 2.368 |
| = | -6.8 | 6.165 | 3.599 |
| = | -6.7 | 4.974 | 2.856 |
| = | -6.7 | 5.678 | 2.898 |
| = | -6.7 | 6.751 | 3.876 |
| = | -6.5 | 18.278 | 16.808 |
| = | -6.5 | 1.994 | 1.759 |

# 5 Conclusions

By clearly connecting the research objective, methodology, and results, this study highlights the potential of molecular docking simulations to unravel mechanisms of drug interaction and resistance, paving the way for more effective targeted therapies in precision oncology. Multiple cancers have been linked to abnormal TRK protein signaling. Opportunities for therapeutic intervention have been revealed following the discovery of mutated NTRK genes as oncogenic factors.

Drugs such as Vitrakvi, which have demonstrated promising early clinical results, could make the target rather than the tumor organ crucial when deciding on a treatment. As a result of technical improvements, more data on gene rearrangements are becoming available, which enables more precise planning of therapeutic interventions to target oncogenic factors. There is reason to be positive for persons who have TRK mutations as well as those in the field of molecularly targeted medications, although clinical trials of TRK inhibitors have just begun.

# Author Contributions

A.A.D.: Conceptualization, formal analysis, research, validation, visualization, writing-original draft, writing-review, and editing.

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BIOTECHNOLOGY

# META-ANALYSIS OF SCIENTIFIC INFORMATION ON THE SPECIES *CARLUDOVICA PALMATA* RUÍZ & PAVÓN

## META-ANÁLISIS DE LA INFORMACIÓN CIENTÍFICA SOBRE LA ESPECIE *CARLUDOVICA PALMATA* RUÍZ & PAVÓN

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**Abstract**

*Carludovica palmata* Ruíz & Pavón is a palm species with great potential in the manufacture of handicrafts, but with limited agricultural management. It is native to Ecuador, and in Mexico it is only distributed in Campeche in the southeast of the country; it serves as raw material for manufacturing Jipi palm hats. The objective of this work is to analyze the spatio-temporal evolution of research published in scientific articles where the species was studied, using bibliometric techniques to identify areas of opportunity in research that have been little developed. We found 78 texts from 1961 to 2022 whose spatio-temporal evolution showed an exponential growth that concentrated in countries of America: Colombia (38), Mexico (11) and Ecuador (8). The most recurrent research topics were botany of the species (20), transformation of its fibers into handicrafts (18), traditional production (*in situ* cultivation) (17) and commercialization of handicrafts (15). However, a null development of propagation techniques of the species was found, a problem that is accentuated if one considers the high demand for its specimens for the manufacture of handicrafts. Therefore, little explored areas of research such as *in vitro* propagation can contribute to the supply of the raw material of an emerging market on products and by-products of the jipi palm. In the case of Mexico, the research focused on the southeast, which coincides with the region where the species is cultivated, but which showed a lack of development in techniques on its propagation.

***Keywords***: Bibliometric analysis, Campeche, iraca palm, jipi palm, jipijapa palm, toquilla straw.

**Resumen**

*Carludovica palmata* Ruíz & Pavón es una especie de palma con amplio potencial en la manufactura de artesanías, pero con limitado manejo agrícola. Es originaria de Ecuador, y en México solo se distribuye en Campeche al sureste del país; sirve como materia prima para la fabricación de los sombreros de palma jipi. El objetivo de este trabajo es analizar la evolución espacio-temporal de la investigación publicada en artículos científicos donde la especie fue objeto de estudio, mediante técnicas bibliométricas para identificar áreas de oportunidad en investigación que han sido poco desarrolladas. Se encontraron 78 textos de 1961 a 2022 cuya evolución espacio-temporal mostró un crecimiento exponencial que se concentró en países de América: Colombia (38), México (11) y Ecuador (8). Los temas de investigación más recurrentes fueron: botánica de la especie (20), transformación de sus fibras en artesanías (18), producción tradicional (cultivo *in situ*) (17) y comercialización de las artesanías (15). Sin embargo, se encontró un nulo desarrollo de técnicas de propagación de la especie, un problema que se acentúa si se considera la alta demanda de sus ejemplares para la fabricación de artesanías. Por lo que áreas de investigación poco exploradas como la propagación *in vitro* pueden contribuir al abastecimiento de la materia prima de un mercado emergente sobre productos y subproductos de la palma jipi. Para el caso de México, la investigación se focalizó en el sureste, que coincide con la región donde se cultiva la especie, pero que evidenció un nulo desarrollo en técnicas sobre su propagación.

***Palabras clave***: Análisis bibliométrico, Campeche, palma iraca, palma jipi, palma jipijapa, paja toquilla.

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# 1 Introduction

*Carludovica palmata* Ruíz & Pavón, also known as *palma jipi* in Mexico, *paja toquilla* or *jipijapa* in Ecuador, and *iraca* in Colombia, is a member of the Cyclanthaceae family cultivated from southern Mexico to northern Bolivia, with a notable presence in Colombia, Panama, and Ecuador (where it is considered native) (Bennett et al., 1992). This palm is valued for its soft, flexible, and durable fibers, which are woven to create hats and other handicrafts (Galviz et al., 2019). These handicrafts are mostly sold in local markets and contribute to the economy of rural producers, for whom the palm has significant cultural importance (Fadiman, 2001).

Several studies have been conducted on *C. palmata*, describing the botany of the species (López et al., 2013; Garcés et al., 2017), its cultural relationship with ethnic groups such as the Quechua in Ecuador (Bennett et al., 1992) and the Maya in Mexico (Fadiman, 2001). Research has also addressed pests and diseases affecting its cultivation (Cordova et al., 2000; Franz and O'Brien, 2001), biotechnology techniques to enhance fiber quality in the transformation process into handicrafts (Ortega et al., 2012; Galviz et al., 2019), and even *in vitro* propagation processes (Hoyos-Sánchez et al., 2020).

However, despite these publications and the cultural and economic significance of the species for agricultural producers and artisans in the regions where *C. palmata* is cultivated, little research has focused on the species as a primary subject of study (Galviz et al., 2019). This phenomenon may largely be due to its status as a local resource with recently gained commercial value, primarily associated with the artisanal production of hats as its sole product (Ortega et al., 2012).

In this context, to understand the research developed around a specific topic and identify opportunities for generating new knowledge, bibliometric studies based on scientific article analysis can be a valuable tool (Cañas-Guerrero et al., 2013). Publishing a scientific study is the most effective way to communicate acquired knowledge resulting from research, and bibliometric analysis can generate indicators to measure the outcomes of scientific and technological activity (Allen et al., 2009). Understanding the scientific information published on a particular topic allows for informed decision-making regarding its improvement and helps identify underexplored research areas (Martínez-Santiago et al., 2017). Bibliometric studies have been applied to species with recently acquired commercial value, such as *Brosimum alicastrum* Swartz (Espinosa-Grande et al., 2023), as well as globally significant crops like maize (Santillán-Fernández et al., 2021), wheat (Giraldo et al., 2019), and rice (Sun and Yuan, 2020).

However, despite the potential of bibliometric studies to bridge knowledge gaps on a particular topic or species, research on *C. palmata* still has unexplored areas where bibliometric analysis could contribute. In this context, the objective of this study is to analyze the spatial-temporal evolution of research published in scientific articles where *C. palmata* was the subject of study, using bibliometric techniques to identify research opportunities that have been insufficiently explored.

# 2 Materials y Methods

## 2.1 Origin of the information

In this study, scientific articles in which the species *C. palmata* was the primary subject of research were considered. Through a content analysis, studies where the species was only mentioned but not analyzed in depth were omitted. The keywords used in the search for scientific articles were *Carludovica palmata* Ruiz & Pavón, *palma iraca*, *palma jipi*, and *paja toquilla*.

Scientific articles available from major publishers (Elsevier, Scopus, and Springer), open-access journal article databases (Conricyt, Scielo, Redalyc, Latindex, Clarivate Analytics, Periodica, and DOAJ), and the freely accessible web search engine Google Scholar were considered. Additionally, the "snowball" technique was applied to retrieve missing articles by reviewing the reference lists of initially identified studies (Leipold, 2014). The scientific articles were collected between January and February 2023, and texts available up to December 2022 were included.

### 2.2 Information analysis

Through a content analysis, the variables evaluated for each scientific article included: author names, year of publication, number of citations, title, text summary, keywords, journal name, institutional affiliation of the first author, country of origin of the first author, and the research area where the study was conducted. The data was recorded in a spreadsheet while maintaining the original language of each text. During data entry, some records were standardized, and special characters were removed or replaced to facilitate analysis, including: "ñ" (changed to "n"), accents, superscripts, subscripts, ®, ©, among others (Aguado-López et al., 2009). Following the methodology of Santillán-Fernández et al. (2021), Espinosa-Grande et al. (2023), and Santillán-Fernández et al. (2023), temporal graphs of scientific production were created using the variables year of publication and number of citations.

Additionally, an ordinary least squares regression model was estimated to determine trends in publication frequency (Gujarati, 2007). Given that the first author bears the primary responsibility for writing and publishing a scientific article (Aguado-López et al., 2009), the countries of origin of the first author were geographically mapped alongside the research areas to identify where research on the *C. palmata* species is being conducted. For this purpose, the geographic software ARGIS ® (ESRI, 2021) was used.

A content analysis of the article titles, abstracts, and keywords was conducted to determine the the matic focus of each text. This classification was ba sed on the system proposed in Scopus (2023) for the *C. palmata* species. Additionally, experts from the Autonomous University of Yucatán (1) and the Postgraduate College, Campeche campus (2) were interviewed. Seven thematic categories were established: (1) Transformation, which included studies associated with the use of the palm in handicraft production; (2) Traditional production, encompassing studies related to the cultivation of the species through conventional agronomic management; (3) *In vitro* production, referring to studies on plant reproduction under artificial environments; (4) Commercialization, comprising studies on producer organization, value addition, sales, distribution, and export of products and by-products derived from the species; (5) Botany, including studies related to the taxonomy of the species; (6) Pests and diseases, focused on the pests and diseases affecting the crop; and (7) Anthropology, addressing the cultural significance of the species in the communities where it is distributed.

Once the scientific articles were categorized by topic, a thematic graph was created based on the first author's country of origin and the temporal distribution of the topics. This aimed to identify potential areas for new research on the *C. palmata* species by country. Finally, bibliometric indicators were generated to identify the most relevant scientific articles, authors, and journals publishing on the species. Using the authors' names, co-authorship networks were constructed in Gephi software (Bastian et al., 2009). Additionally, the variables first author's country, first author's institutional affiliation, research areas, and citations were analyzed to determine the most relevant scientific articles based on citation count, as well as the institutions and research areas by country that have contributed to knowledge on the species. In the case of Mexico, all institutions (of both first authors and collaborators) conducting research on the species were spatially mapped alongside the regions where it is cultivated.

## 3 Results and Discussion

### 3.1 Spatiotemporal analysis

From 1961 to 2022, a total of 78 scientific articles were published in which the *C. palmata* species was the subject of study. This body of scientific work generated 356 bibliographic citations (Figure 1). The first recorded study dates back to 1961. However, from 2001 onward, there was a growing trend in research on the species. The most productive period was from 2001 to 2021, accounting for 76.92% of the total publications (60), which contributed to an exponential growth trend in publications ($R^2$ = 0.2785). The most cited works were those published between 1961 and 2001, collectively accounting for 61.80% of total bibliographic citations (220).

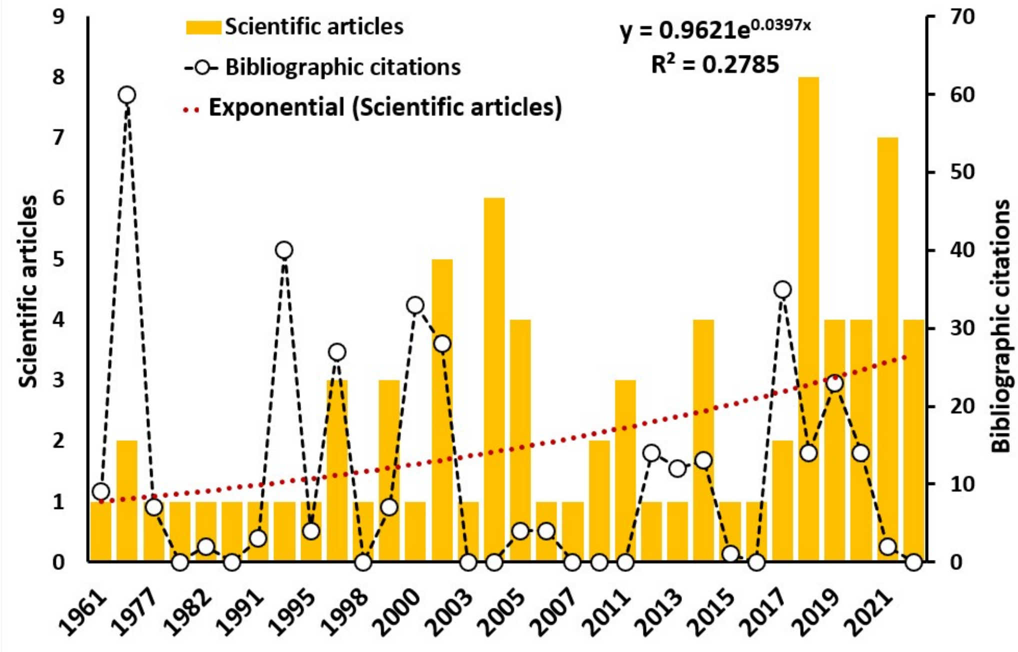


**Figure 1.** Temporal evolution of scientific production and bibliographic citations where the *C. palmata* species was the subject of study from 1961 to 2022.

Poot-Pool et al. (2018) attribute this increase in the number of publications to the recent commercial value of the species as a raw material for regional handicrafts in Latin America, where it originates. This commercial value has made *C. palmata* a recurrent research topic in southeastern Mexico, Central America, and South America, as it serves as a local resource that supports the economic development of rural regions where it is found (Galviz et al., 2019). However, Ortega-Haas et al. (2020) found that further research is needed on the cultivation of the species to ensure a sustainable supply of raw material for the economic activities that depend on it.

Based on the country of origin of the first author of the scientific articles, the 78 studies originated from 12 countries: Colombia (48.72 %, 38 texts), Mexico (14.10 %, 11), Ecuador (10.26 %, 8), the United States of America (7.69 %, 6), Costa Rica (6.41 %, 5), Peru (5.13 %, 4), Germany (1.28 %, 1), Austria (1.28 %, 1), Brazil (1.28 %, 1), Canada (1.28 %, 1), Spain (1.28 %, 1), and Nicaragua (1.28 %, 1) (Figure 2). Figure 2 shows that the majority of research on *C. palmata* has been conducted in Latin American countries (68 texts, 87.18 %). Additionally, the study areas of the 78 analyzed articles were geographically located within this region of the Americas. This is explained by the fact that the species' center of origin is in the province of Manabí, Ecuador (Ruiz and Pavón, 1798), from where it has spread to southeastern Mexico, Central America, and the Amazon region in South America (Bristol, 1961).

The research studies that contributed the most knowledge about the *C. palmata* species were: Botany (20 texts, 25.64 %), Transformation (18, 23.09 %), Traditional production (17, 21.79 %), and Commercialization (15, 19.23 %), collectively representing 89.75 % (70 texts) (Table 1). However, during the analysis period (1961–2022), it was observed that although the earliest studies focused on the Botany of the species (1961), it was not until 1978 that scientific articles on transformation and commercialization emerged. From 1987 onward, publications related to cultivation began to appear, followed by studies on agronomic management in 2000. Additionally, it is noteworthy that the first research on propagation techniques in artificial environments (*In Vitro* Production) appeared in 2020.

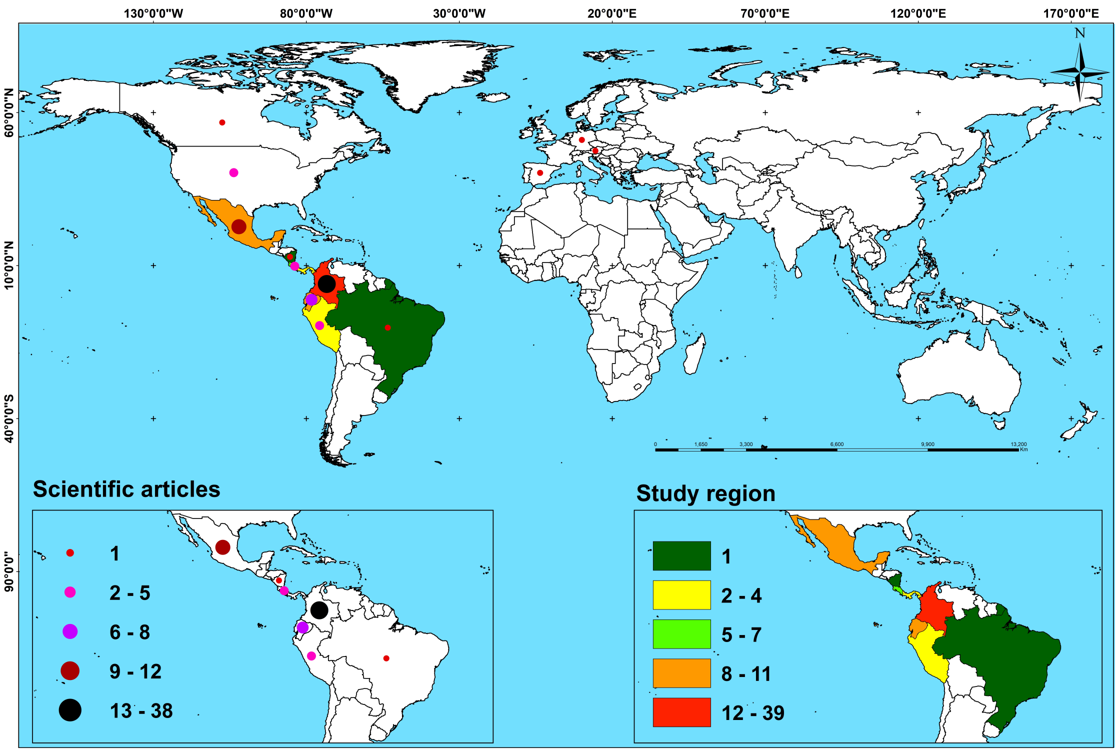


**Figure 2.** International spatial distribution of study areas and scientific text productivity where the *C. palmata* species was analyzed from 1961 to 2022.

In nine of the twelve countries that conducted research on *C. palmata*, the topic of Botany was recurrent (Figure 3). In contrast, topics related to its traditional cultivation were only found in four countries (Colombia, Mexico, USA, and Canada), with Colombia and Mexico being the only countries where *in situ* cultivation has been reported (Ortega-Haas et al., 2020). Regarding studies on the *in vitro* propagation of the species, only Colombia has reported advancements in this area. Therefore, in the case of Mexico, topics such as *in vitro* propagation, agronomic management of the species, and commercialization models remain unexplored areas. According to Espinosa-Grande et al. (2023), when the uses of a local species are enhanced, the main challenge is to conduct research on its sexual or asexual reproduction to ensure a stable supply of raw materials for the economic activities developed around the species.

**Table 1.** Timeline of research topics developed around the *C. palmata* species from 1961 to 2022.

| Topic | Scientific Articles | | Period |
|---|---|---|---|
| | Number | % | |
| Botany | 20 | 25.64 | 1961-2022 |
| Transformation | 18 | 23.09 | 1978-2021 |
| Traditional Production | 17 | 21.79 | 1987-2022 |
| Commercialization | 15 | 19.23 | 1978-2021 |
| Anthropology | 4 | 5.13 | 1992, 2001 |
| Pests and Diseases | 2 | 2.56 | 2000-2001 |
| *In Vitro* Production | 2 | 2.56 | 2020, 2022 |
| Total | 78 | 100 | 1961-2022 |

## 3.2 Bibliometric Indicators

Of the 78 scientific articles, 79.49% (62 texts) were published in Spanish, while 20.5% (16) were in English. Among the 356 bibliographic citations, 24.44% (87) corresponded to texts published in Spanish, whereas 75.56% (269) were from texts in English. According to Li and Zhao (2015), English has been adopted as the universal language of the scientific community, making publications in English more likely to be disseminated internationally. In fact, eight of the ten most cited scientific articles were published in English (Table 2). Collectively, these eight articles accounted for 62.08% of the total bibliographic citations (221) and were mostly published in journals with impact factors above 3. Santillán-Fernández et al. (2021) found that the impact factor of journals increases the probability of reaching a wider audience.

Regarding the research topics covered in the ten most cited articles, studies focusing on the cultural importance of *C. palmata* in indigenous communities in Ecuador (Quechua) and Mexico (Maya) stood out. Additionally, studies on pests and diseases were prominent, with research conducted in countries where the species is cultivated *in situ* (Mexico and Ecuador). Consequently, cultural aspects and agronomic management of cultivation present opportunities for generating new knowledge. Table 2 also shows that five of the ten most cited studies were conducted by authors whose country of origin was different from the study area (USA: 4, Canada: 1).

Table 3 reveals that in the countries where *C. palmata* is naturally distributed (Colombia, Mexico, Ecuador, Costa Rica, Peru, Nicaragua, and Brazil), research institutions conducting studies on the species geographically locate their research areas within their own territory. This does not occur in European countries (Germany, Austria, and Spain), the USA, or Canada. Gersbach and Schneider (2015) found that economically developed countries such as the USA and European nations invest more in their research centers, enabling them to conduct studies beyond their national borders. Additionally, Espinosa-Grande et al. (2023) observed that in studies on locally significant species, the production of new knowledge by researchers from outside the study areas is common. Therefore, strengthening international co-authorship networks presents a viable strategy for generating new knowledge in regions of interest through external investments (Aguado-López et al., 2009).

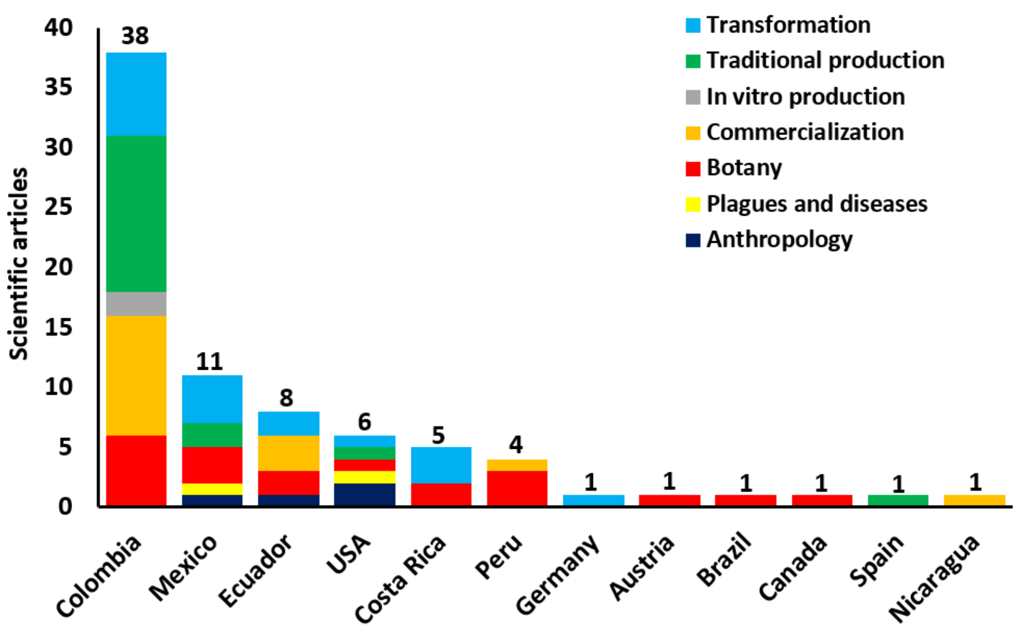


**Figure 3.** Research topics of the main nations that published scientific articles where the *C. palmata* species was studied from 1961 to 2022.

**Table 2.** Bibliometric indicators of the main scientific articles where the species *C. palmata* was the subject of study from 1961 to 2022, ordered according to the number of bibliographic citations.

| First author | | | Scientific journal | | | | Topic | Area of Study |
|---|---|---|---|---|---|---|---|---|
| **Author** | **Institution** | **Country** | **Name** | **JCR (WoS, 2021)** | **Languaje** | **Citations** | | |
| Bennett et al. (1992) | Jardín Botánico de NY | USA | Economic Botany | Q2 / 2.6 | English | 35 | Anthropology | Ecuador |
| Cordova et al. (2000) | CICY | Mexico | Plant Disease | Q1 / 4.6 | English | 37 | Pests and diseases | Mexico |
| Garcés et al. (2017) | PUCE | Ecuador | Frontiers in Microbiology | Q1 / 6.1 | English | 37 | Botany | Ecuador |
| Wilder (1976) | Universidad de Illinois | USA | American Journal of Botany | Q1 / 3.3 | English | 34 | Botany | Panama |
| Anderson and Gomez (1997) | MuCaNa | Canada | Revista de Biología Tropical | Q3 / 0.8 | English | 21 | Botany | Costa Rica / Panama |
| Galviz et al. (2019) | UNC-Medellín | Colombia | Food and Bioproducts Processing | Q1 / 5.1 | English | 27 | Transformation | Colombia |
| Franz and O'Brien (2001) | Universidad de Cornell | USA | A_ Entomological _SA | Q2 / 2.7 | English | 20 | Pests and diseases | Costa Rica / Panama / Ecuador |
| Ortega et al. (2012) | Liceo Bella Suiza | Colombia | UDCA | No tiene | Spanish | 14 | Transformation | Colombia |
| López et al. (2013) | UPS | Ecuador | Ingenius | No tiene | Spanish | 12 | Botany | Ecuador |
| Fadiman (2001) | Universidad de Texas | USA | Economic Botany | Q2 / 2.6 | English | 10 | Anthropology | Mexico |

NY: New York; CICY: Centro Investigaciones Científicas de Yucatán; PUCE: Pontificia Universidad Católica del Ecuador; MuCaNa: Canadian Museum of Nature; UNC-Medellín: Universidad Nacional de Colombia Sede Medellín; UPS: Universidad Politécnica Salesiana; UDCA: Revista UDCA Actualidad & Divulgación Científica; Ingenius: Revista de Ciencia y Tecnología; A_ Entomological _SA: Annals of the Entomological Society of America; USA: United States of America.

**Table 3.** Association of the main research institutions and their study areas by country, which have published scientific articles where the *C. palmata* species was the subject of study.

| Country | Institutions | Study Area |
|---|---|---|
| Colombia (38) | UNC-Medellín (13) | Nariño, Colombia (8) |
| | Universidad de Antioquia (9) | Usiacurí, Colombia (7) |
| | Universidad de Nariño (8) | Medellín, Colombia (5) |
| | PU-Javeriana (4) | Chocó, Colombia (4) |
| | Colegio Liceo bella Suiza (1) | Bolívar, Colombia (4) |
| | Universidad de Córdoba (1) | Boyacá, Colombia (3) |
| | UC-Barranquilla (1) | Sucre, Colombia (3) |
| | Universidad de los Andes (1) | Lorica, Colombia (3) |
| | | Huila, Colombia (1) |
| Mexico (11) | CICY (4) | Campeche, Mexico (11) |
| | IT-Chiná (2) | |
| | ECOSUR-Cam (2) | |
| | IT-Mérida (1) | |
| | ITS-Calkiní (1) | |
| | UA-NLeón (1) | |
| Ecuador (8) | Universidad Politécnica Salesiana (5) | Manabí, Ecuador (5) |
| | PUCE (2) | Cuenca, Ecuador (3) |
| | Universidad del Azuay (1) | |
| USA (6) | Universidad de Illinois (1) | Mexico (1) |
| | Jardín Botánico de NY (1) | Colombia (2) |
| | Universidad de Cornell (1) | Costa Rica (1) |
| | Universidad de Texas (1) | Panama (1) |
| | Universidad de Harvard (1) | Ecuador (1) |
| | Universidad Estatal de Cleveland (1) | |
| Costa Rica (5) | CATIE-CR (3) | Puntarenas, Costa Rica (3) |
| | Universidad de Costa Rica (1) | Cartago, Costa Rica (2) |
| | Universidad Nacional de Costa Rica (1) | |
| Perú (4) | UNCP (2) | Ucayali, Perú (3) |
| | UNIA (2) | Lima, Perú (1) |
| Nicaragua (1) | UNA-Camoapa | Nicaragua (1) |
| Germany (1) | Universidad de Ulm (1) | Guyana Francesa (1) |
| Austria (1) | Universidad de Viena (1) | Costa Rica (1) |
| Brazil (1) | Universidad Estatal Paulista (1) | Brasil (1) |
| Canada (1) | Museo canadiense de la naturaleza (1) | Costa Rica / Panama (1) |
| Spain (1) | Universidad Politécnica de Valencia (1) | Ecuador (1) |
| Total (78) | 35 | 9 |

**Barranquilla**: Universidad de la Costa Barranquilla; **CICY**: Centro de Investigaciones Científicas de Yucatán; **IT-Mérida**: Instituto Tecnológico de Mérida; **UA-Chapingo**: Universidad Autónoma Chapingo; **IT-Chiná**: Instituto Tecnológico de Chiná; **ECOSUR-Cam**: El Colegio de la Frontera Sur, Campeche campus; **ITS-Calkiní**: Instituto Tecnológico Superior de Calkiní; **UA-NLeón**: Universidad Autónoma de Nuevo León; **PUCE**: Pontificia Universidad Católica del Ecuador; **NY**: New York; **CATIE-CR**: Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica campus; **UNCP**: Universidad Nacional del Centro de Perú; **UNIA**: Universidad Nacional Intercultural de la Amazonía; **UNA-Camoapa**: Universidad Nacional Agraria Camoapa.

In the case of Mexico, it was found that most institutions or research centers that have studied the *C. palmata* species are geographically located in the southeast of the country (eight out of eleven institutions). Additionally, in all cases, the study area referenced has been the municipality of Calkiní in Campeche (Figure 4). Espinosa-Grande et al. (2023) found that for species with recent commercial value, it is common for new knowledge to be developed by institutions located within the region where the species is cultivated or naturally distributed. According to Santillán-Fernández et al. (2021), this facilitates the transfer of technology to agricultural producers.

## 4 Co-authorship network

In the 78 analyzed texts, 63 different first authors were identified. Including first authors and co-authors, the total number of unique individuals involved was 112. The author and co-author network (Figure 5) consisted of 112 nodes (authors) and 90 edges (connections). In co-authorship network analysis, connections are crucial because they enable an author to access ideas, knowledge, and information that would otherwise be socially distant to them (Granovetter, 1973). The main authors conducting research on *C. palmata* from 1961 to 2022 were: Galviz_Quesada_A (4 texts) from Universidad Nacional de Colombia, Medellín (UNC-Medellín), focusing on biotechnology applied to species transformation processes; Zambrano_Arteaga_JC (3 texts, Fundación Universitaria Navarra), Chicaiza_Finley_D (3, UNC-Medellín), and Hoyos_Sanchez_RA (3, UNC-Medellín), mainly researching biotechnology applied to *in vitro* propagation of the species.

Another author in the network is Lopez_L (3 texts) from Universidad Politécnica Salesiana, Ecuador, who has also applied biotechnology to enhance the transformation of the species into commercially valuable products. Figure 5 further highlights a subnetwork composed of researchers from Mexico: Cordova_I (CICY), CetzalIx_W (IT-Chiná), Ortega_Hass_JJ (ECOSUR-Campeche),Godoy_Hernandez_G (CICY),Munoz_Sanchez_A (CICY), and Gonzalez_Estrada_T (CICY). Similar to their counterparts in Colombia and Ecuador, these researchers have applied biotechnological methodologies to revalorize the uses of the species.

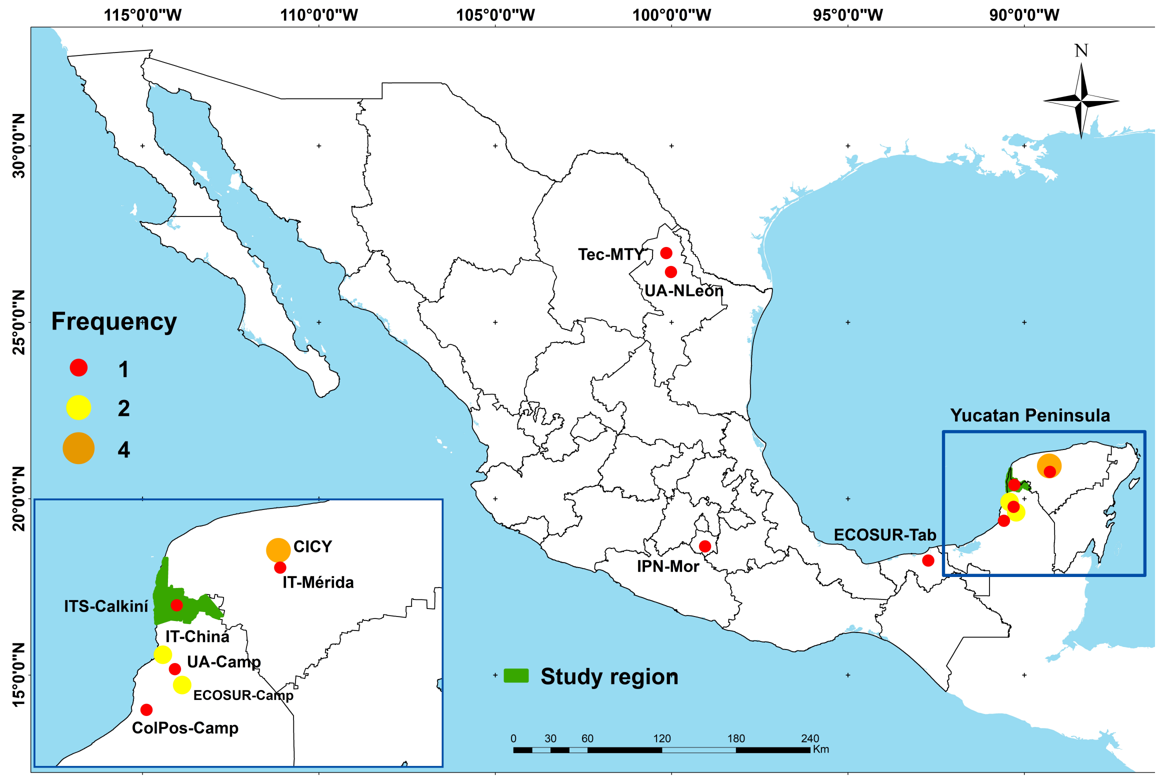


**Figure 4.** Spatial distribution of research institutions in Mexico that published scientific articles where the *C. palmata* species was studied from 1961 to 2022.

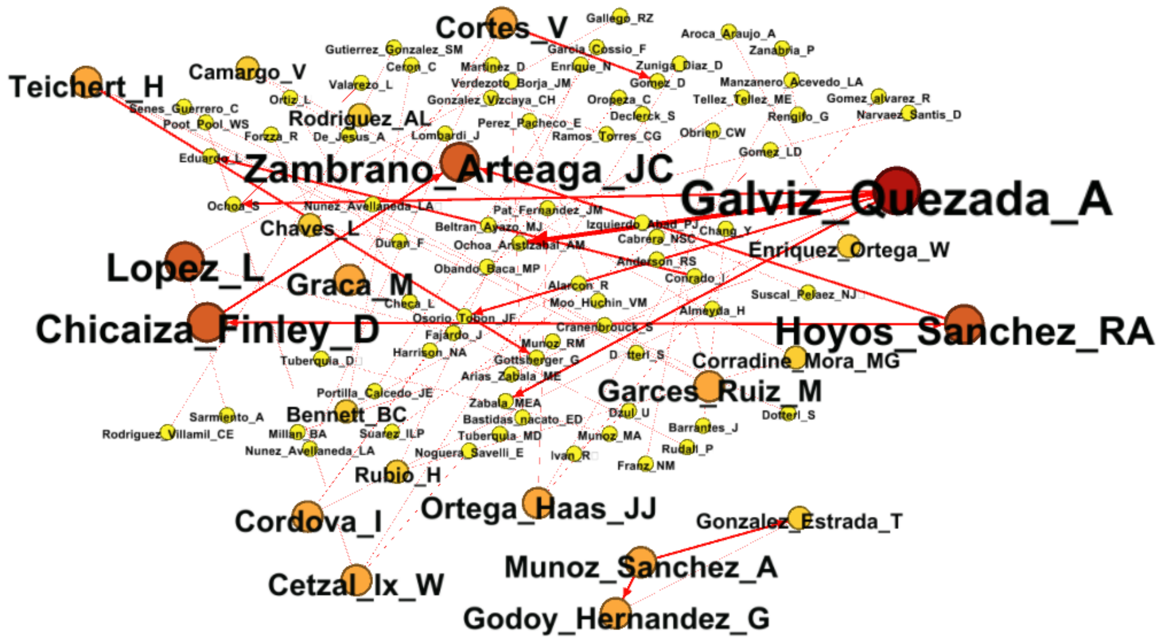


**Figure 5.** International network of authors and collaborators who conducted research on the *C. palmata* species from 1961 to 2022. The size of the node corresponds to productivity.

Santillán-Fernández et al. (2023) found that in Mexico's non-timber forest sector, biotechnology presents an opportunity-especially for species of recent commercial value. Most research has focused on describing the botany and uses of species, leaving knowledge gaps on how to improve the quality of raw materials (Espinosa-Grande et al., 2023).

The density of the co-authorship network was 0.002, revealing low collaboration between authors from different countries. Network density measures how well nodes (authors) interact with one another. It ranges from 0 to 1, where values closer to 1 indicate stronger interactions (Aguilar-Gallegos et al., 2016). However, in Colombia and Mexico, researchers from the same institution tend to collaborate among themselves.

According to Silva et al. (2014), this restricts constructive criticism and limits feedback on research relevance. Institutional research groups often replicate the same methodologies in different study areas, which hinders innovation and increases redundancy in published research (Santillán-Fernández et al., 2023). Thus, creating synergies with authors from other institutions could be a valuable strategy to improve both the quantity and quality of research on *C. palmata* at the national and international levels.

## 5 Conclusions

The spatio-temporal evolution of scientific production revealed an exponential increase in scholarly publications addressing *C. palmata* as a research topic between 1961 and 2022. This output was concentrated in American countries where the species occurs naturally: Colombia (38), Mexico (11), and Ecuador (8). The most recurrent research themes were species botany (20), transformation of its fibers into handicrafts (18), traditional production (*in situ* cultivation) (17), and commercialization of handicrafts (15).

However, the studies with the greatest impact (measured by the number of citations) were predominantly conducted by researchers from the United States and Canada. Although these investigations were carried out in Latin America, they were published in high-impact journals in English.

Consequently, research on *C. palmata* in Latin America still has substantial room for improvement through the publication of studies in English and in higher-impact journals. A research gap was identified regarding the management and propagation of the species in artificial environments (*in vitro*). Except for Colombia, no other country has published studies on this subject. In the case of Mexico, research on *C. palmata* was concentrated in the southeastern region and led by institutions such as CICY, IT-Chiná, and ECOSUR-Campeche. These research centers are geographically close to the natural distribution area of the species, which could be a key success factor in generating impactful new knowledge, as it facilitates technology transfer. This is particularly relevant considering that research on C. palmata is still in its early stages.

Additionally, it was found that authors from the same institution tend to collaborate mainly among themselves, which limits constructive criticism and reduces feedback on research relevance. Strengthening collaborations with authors from other institutions could be an effective strategy to enhance both the quantity and quality of research on the species.

Finally, bibliometric techniques proved to be a useful methodology for identifying areas of opportunity in the development of new knowledge on *C. palmata*. Nevertheless, the theoretical nature of the findings should be acknowledged, as they primarily contribute to broadening the state of the art. Therefore, future studies are encouraged to focus on the practical application of scientific findings to agricultural management of the crop and to the conservation of the species.

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## Authors' contribution

L.A.P.H.: Data curation. A.S.F.: Conceptualization, Formal analysis, Methodology, Writing – review and editing. N.A.G.: Writing – original draft. J.C.A.M.: Supervision. T.A.G.E.: Supervision. J.H.C.V.: Project administration.

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# Study of agricultural biomass waste for the installation of a small-scale biorefinery

## Estudio de residuos biomásicos agrícolas para la instalación de una biorrefinería de pequeña escala

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**Abstract**

In recent years, lignocellulose residues have been used for producing different bioproducts. Among the countries with this potential is Ecuador, which is characterized by having an agrarian economy, generating waste that constitutes mostly biomass of the lignocellulosic type. The province of Manabí, located on the Ecuadorian coast, is an agricultural area whose residues are usually burned, left in the field or used for animal feed. Based on this premise, a multicriteria analysis was developed through the Analytical Hierarchy Process (AHP), in which 6 agricultural biomasses from the Ecuadorian coast were evaluated: coffee husks, cocoa husks and mucilage, corn cob, banana peels and sugar cane bagasse. They were evaluated by a panel of experts based on their energy potential, biomass composition, yield, processing cost and environmental impact. The composition of the waste (proximal and elemental analysis) was bibliographically consulted, as well as current processing technologies. From the AHP, it was known that the agricultural biomass with the greatest potential to be used in a small-scale biorefinery is sugarcane bagasse (33.20 %), followed by coffee husks (26.10 %), being the recognized sugarcane with the greatest richness in polysaccharides and a promising source for obtaining biofuels and other chemical products. It is expected that the results obtained in this study will be the basis for other research and will be interesting for the bioeconomic development of the country.

***Keywords***: Biomass, biorefinery, Ecuador, biomass, analytical hierarchy process, agricultural wastes.

**Resumen**

En los últimos años se ha considerado el uso de residuos lignocelulosos para la producción de distintos bioproductos. Entre los países con este potencial está el Ecuador, el cual tiene una economía agraria, generadora de residuos que constituyen en su mayoría biomasa lignocelulósicas. La provincia de Manabí ubicada en la costa ecuatoriana es una zona agrícola cuyos residuos son por lo general quemados, dejados en el campo o usados para alimentación animal. Atendiendo a esta premisa, se desarrolló un análisis multicriterios a través del proceso de jerarquía analítica (AHP), en el cual se evaluaron 6 biomasas agrícolas: cascarilla de café, cáscara y mucílago de cacao, olote de maíz, cáscara de plátano y bagazo de caña de azúcar. Los mismos fueron ponderados por un panel de expertos en función a su potencial energético, composición, rendimiento de biomasa, costo de procesamiento e impacto ambiental. Para ello se consultó bibliográficamente la composición de los residuos (análisis proximal y elemental), así como las tecnologías actuales de procesamiento. A partir del AHP se conoció que la biomasa agrícola con mayor potencial de ser utilizado en una biorrefinería de pequeña escala es el bagazo de caña de azúcar (33,20 %), seguido de la cascarilla de café (26,10 %), siendo la caña de azúcar reconocida con mayor riqueza en polisacáridos y una fuente prometedora para la obtención de biocombustibles y otros productos químicos. Se espera que los resultados obtenidos sean de fundamento para otras investigaciones y de interés para el desarrollo bioeconómico del país.

***Palabras clave***: Biomasa, biorrefinería, Ecuador, biomasa, proceso de jerarquía analítica, residuos agrarios.

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# 1 Introduction

The global economy is based on the commercialization of products and the reliance on non-renewable resources such as oil (Navarrete Loza and Saavedra Cuadrado, 2014). Although this has been the predominant business model, many countries are currently striving to transition from a traditional economy to a bioeconomy, in alignment with the goals set by the 2030 Sustainable Development Goals (SDGs).

Bioeconomy is defined as the use of biological resources (biomass), innovative biological processes, and principles to sustainably produce goods and services (Birner, 2018). Biomass is understood as biological-origin material derived from living or once-living organisms, excluding those embedded in geological formations or fossilized (Birner, 2018). Biomass that can potentially be used as feedstock in biorefineries (industrial complexes where biomass is processed) can come from various sources, including agriculture, forestry, domestic organic waste, and microalgae, among others (Hernández Benítez and Céspedes Rangel, 2019).

The term biorefinery emerged in the 1990s, based on the concept of Chemurgy, as an attempt to produce a variety of biobased products using agricultural biomass as raw material (Pazmiñon Sánchez et al., 2017). The U.S. National Renewable Energy Laboratory (NREL) highlights that biorefineries are facilities that integrate biomass conversion processes and equipment to produce a wide range of products, including fuels, energy, and chemicals (Redondo-Gómez et al., 2020). These facilities aim to add value to a diverse range of renewable raw materials, including biomass from forestry, aquaculture, and agricultural waste, such as those derived from crop and livestock activities (Katakojwala and Mohan, 2021).

First-generation biorefineries in developed countries such as Belgium, the Netherlands, France, Austria, and Germany report the use of starch or forage from wheat and corn—both human-consumable raw materials—for the commercial production of bioethanol (Gutiérrez Villanueva et al., 2020). Additionally, agricultural residues, such as bagasse, rice straw, and corn stover, are used as raw materials in the pulp and paper industry (Mongkhonsiri et al., 2018). Countries like Brazil employ sugarcane biomass for biofuel production (Pazmiñon Sánchez et al., 2017). At a global scale, studies have reported the use of various agricultural residues, such as rice straw, corn husks and stover, and pineapple waste for bioethanol production (Kumar et al., 2018; Kazemi Shariat Panahi et al., 2020; Chintagunta et al., 2017), as well as orange peels for bioplastics production (Gutiérrez Villanueva et al., 2020).

These agricultural biomasses are classified as lignocellulosic residues, and, in addition to being a renewable substrate in comparison to fossil fuels, they are primarily composed of three constituents: cellulose, hemicellulose, and lignin (Sharma and Saini, 2020). Cellulose is the most abundant organic polymeric material on Earth and is commonly used for cardboard, paper production, and as a precursor for second-generation bioethanol (Yousuf et al., 2020; Korányi et al., 2020). Hemicellulose is an amorphous, branched heteropolysaccharide composed of five- and six-carbon sugars. The presence of reducing sugars in hemicellulose is crucial as a key source for chemical production (Lorenci Woiciechowski et al., 2020; Mankar et al., 2021).

Lignin, another important component of lignocellulosic biomass, is a complex aromatic biopolymer with a high carbon content. Despite being underutilized in biomass processing, lignin holds significant potential as a raw material for the chemical and fuel industries (Mathew et al., 2018; Korányi et al., 2020). Due to their composition, lignocellulosic residues can be used to produce high-value-added bioproducts, such as lactic acid, furfural, and levulinic acid (Espinoza-Vázquez et al., 2020).

The interest in using lignocellulosic residues, especially agricultural ones, lies in their low cost and abundance of compounds suitable for lignocellulosic biorefineries. Studies report that small-scale biorefineries have used agricultural residues for the production of biogas, xylan, glucose, ethanol, and polyhydroxyalkanoates (Parralejo et al., 2019; Dos Santos et al., 2017; Clauser et al., 2018).

In countries such as Ecuador, biomass is abundant due to its megadiverse ecosystem and extensive agricultural activity. In 2020, the country recorded 5.20 million hectares of cultivated land, with sugarcane, bananas, and African palm as the primary crops (INEC, 2020). Manabí is one of Ecuador's provinces with the highest number of agriculture-related economic activities, covering 1.2 million hectares of farmland, which accounts for 15.83 % of the national territory. Additionally, the province hosts small-scale agro-industrial processing centers, including snack producers, banana flour mills, rice and corn processors, and sugarcane and coffee industries, responsible for producing sugarcane, aguardiente, roasted and ground coffee (Manabí Produce, 2021; Manabí Produce-Ep, 2016).

This agricultural and agro-industrial activity generates residues that are characterized as potentially renewable, sustainable, cost-effective, and economically viable resources for bioenergy production (Gupta and Verma, 2015). Therefore, agricultural, livestock, and urban waste byproducts can be utilized in small-scale biorefineries (Gómez-Soto et al., 2019). Since biorefineries rely on residual biomass, they contribute to reducing energy costs and greenhouse gas emissions, while simultaneously producing energy, materials, and chemicals (Carmona-Cabello et al., 2018).

By the time the research was carried out, besides the sugar mills, there is only one biorefinery in Ecuador, specifically at the Nayón campus of the Pontifical Catholic University of Ecuador. This facility is currently operational, developing products such as ethanol, biogas, biofertilizers, fuel, animal feed, and other high-value chemical compounds (Carvajal, 2013; Cevallos, 2018). However, no industrial complex of this kind has been registered in the province of Manabí, despite the large amount of agricultural waste generated in the region (Sumba et al., 2019). The implementation of a small-scale biorefinery in Manabí would enhance agricultural activities by utilizing waste that currently has a negative environmental impact.

The use of agricultural residues as raw materials presents a promising option to increase economic value while positively impacting the province's economy and the environment. Such a decision requires multicriteria analysis to assess different alternatives and select the optimal one. Various decision-making tools exist, among which stands out the Analytic Hierarchy Process (AHP).

AHP is recognized as a comprehensive evaluation method for renewable energy sources, providing the foundation for proper decision-making by assessing the potential of biomass utilization from a multicriteria perspective (Jiménez Borges et al., 2019). The selection of biomass using AHP has been tested in studies, such as evaluating the sustainability of major biomasses in Cienfuegos, Cuba, where bagasse showed the highest energy contribution (Jiménez Borges et al., 2019). Another application of AHP was in systematically determining the best agricultural residue for polyhydroxyalkanoate production (Requiso et al., 2018).

Within this context, it is innovative to apply the AHP method to evaluate the use of agricultural by-products in a specific area of Ecuador, with the aim of being used as raw material in a small-scale biorefinery.

Thus, this study aims to review agricultural biomass generated in the province of Manabí and apply an Analytic Hierarchy Process to select the most suitable biomass to be used in a small-scale biorefinery. A comprehensive analysis of this process and its results will serve as a framework for bioeconomy development in Ecuador.

## 2 Materials and Methods

### 2.1 Multi-criteria analysis method

To select the by-products with the highest importance index, the Analytic Hierarchy Process (AHP) was used. This is a decision analysis method developed by Thomas Saaty in 1980, designed to facilitate decision-making by providing a structured approach for determining the weights and priorities of multiple criteria, standardizing them for comparison (Ramírez et al., 2020). The application of the AHP method follows these steps (Huamaní Huamaní and Eyzaguirre Tejada, 2015):

- Goal Selection: This involves defining the objective to be achieved. It requires access to expert knowledge on the topic to select criteria and propose alternatives.

- Hierarchical Structure: Once the overall goal, criteria, and solution alternatives are defined, a hierarchical model is proposed.

- Matrix Proposal: This step involves making pairwise comparisons using a numerical scale that corresponds to commonly used verbal expressions (Table 1).

The iterative process was repeated for each criterion until the results were obtained and subsequently subjected to a consistency measure. The consistency index, $\lambda_{max}$, was determined as a primary eigenvalue based on the eigenvector technique. This was achieved by calculating the multiplication capacity of the matrix of criterion ratings (in the row of the pairwise comparison matrix) and the normalized average of all components (within the column of the normalized matrix), divided by the normalized average of the criterion (Owolabi et al., 2020). Subsequently, the Consistency Index (CI) was calculated (Luna et al., 2019), using Equation 1.

$$CI = \frac{(\lambda_{max} - n)}{(n-1)} \tag{1}$$

Where $n$ represents the matrix size, and $\lambda_{max}$ is the maximum eigenvalue. Then, using Equation 2, the Consistency Ratio (CR) is calculated, which compares the Consistency Index (CI) of the matrix derived from the judgments with the Random Consistency Index (RI) of a random matrix (Table 2).

$$RC = \frac{CI}{RI} \tag{2}$$

If the Consistency Ratio (CR) exceeds 0.10, it indicates that the judgments within the matrix are inconsistent and, therefore, unacceptable for decision-making. Conversely, if CR values are equal to or less than 0.10, the consistencies are deemed acceptable, valid, and justified for selecting alternatives.

**Table 1.** Saaty's Scale

| Numerical scale | Verbal scale |
|---|---|
| 1 | Equally important. |
| 3 | The element is moderately more important with respect to the other. |
| 5 | The element is strongly more important with respect to the other. |
| 7 | The importance of the element is very strong with respect to the other. |
| 9 | The importance of the element is extreme with respect to the other. |
| 2, 4, 6, 8 | Intermediate values between two adjacent judgments. |
| Increments 0, 1 | Intermediate values between increments (use this scale if you think your assessment needs a high degree of precision). |

Source: (Saaty, 2014)

## 2.2 Selection alternatives

The selection alternatives represented each of the agricultural by-products considered in the study. These were chosen based on data reported by the National Institute of Statistics and Censuses (INEC, 2020), highlighting the top agricultural activities in terms of production in Manabí, Ecuador, in 2020. Consequently, the study focused on waste from coffee (husk), cocoa (shell and mucilage), corn (cob), banana (peel), and sugarcane (bagasse), as their constituents represent a promising source of lignocellulosic material. Additionally, a literature review was conducted to determine the proximate composition (% moisture, volatile solids, ash, and fixed carbon) and elemental composition (carbon, nitrogen, hydrogen, oxygen, and sulfur) of the evaluated residues. Furthermore, the study considered current biomass processing methods as well as the various products that can be derived from them.

**Table 2.** Consistency index

| **Matrix size (n)** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Random Index** | 0 | 0 | 0.58 | 0.90 | 1.12 | 1.24 | 1.32 | 1.41 | 1.45 | 1.49 |

Source: (Qazi et al., 2018)

### 2.3 Selection criteria for alternative evaluation

Agricultural residual biomass has the potential to be converted into syngas for energy generation or into a range of basic chemicals derived from compounds such as cellulose, hemicellulose, starch, lignin, lipids, and proteins. The conversion of biomass into chemicals presents diverse opportunities but also poses technological challenges due to its complex structure, which requires pretreatment processes to break down the material into monosaccharides for high-value product generation (Kover et al., 2021). Based on these considerations and previous research on the subject, five selection criteria were established:

- **Energy potential:** Biomass is a renewable energy resource that not only provides clean energy but also ensures long-term energy availability if used sustainably (Morato et al., 2019). This criterion assesses the potential of agricultural residues to be converted into energy.

- **Composition:** The chemical composition of lignocellulosic biomass determines its suitability as a biorefinery feedstock, considering factors such as cellulose, hemicellulose, lignin, and proximate and elemental content (Jaffar et al., 2020).

- **Biomass yield:** Refers to the harvestable quantity of biomass obtained during the production cycle of a given crop (Cobuloglu and Büyüktahtakin, 2015).

- **Processing cost:** Measures the cost of converting biomass into usable products or energy. Biomass can undergo various processing methods depending on the desired end products, utilizing chemical, thermal, thermochemical, and biochemical conversion technologies (Shahbaz et al., 2020).

- **Environmental impact:** Agricultural biomass has been identified as a potential alternative to reduce fossil fuel dependence and mitigate environmental damage (Fantini, 2017). This criterion evaluates the environmental benefits associated with selecting a specific type of residue.

The availability of biomass depends on the production volume and seasonality of each crop. However, these aspects were not included as selection criteria, as the study focused on evaluating the potential of the generated residues to be used in biorefining processes aimed at producing various bioproducts.

### 2.4 Expert panel

To determine the weight of the selected criteria, expert opinions were sought from a panel of 14 professionals with direct expertise in the subject matter. It is recommended that such panels include 7 to 15 participants to maintain high confidence levels and reliable evaluations (Gómez Montoya et al., 2008). Additionally, a Google Forms questionnaire was structured based on the AHP method, allowing each expert to assess the agricultural by-products according to the established selection criteria to be used as feedstock in a small-scale biorefinery. Once the expert panel evaluated and weighted the selection criteria, a consensus was reached for AHP implementation.

### 2.5 Software Tools Used

The study employed Super Decisions V3.2, a free educational software developed by Saaty for Analytic Network Processes (ANP) and Analytic Hierarchy Processes (AHP).

## 3 Results and Discussion

Based on the reviewed literature, the lignocellulosic composition, as well as the proximate and elemental content of the evaluated residues, was determined (Table 3). Depending on their composition, these residues can serve as raw materials for the production of textiles, packaging, steroids, pulp and paper, construction materials, fertilizers, and animal feed. Lignocellulosic materials have applications in both animal and human consumption products. Previous studies have demonstrated their use in the production of alcohol, xylose, xylitol, and xylo-oligosaccharides (Muñoz-Muño et al., 2014).

**Table 3.** Lignocellulosic, proximal and elemental composition of the analyzed wastes

| Biomass | Cel (%) | Lig (%) | Hemic (%) | Hum (%) | SV (%) | CF (%) | Cn (%) | C (%) | N (%) | H (%) | O (%) | S (%) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Coffee husk** | 24.50 | 23.70 | 29.70 | 11.30 | 72.94 | 7.76 | 8.00 | 39.68 | 3.01 | 5.41 | 51.58 | 0.32 | (Murthy and Madhava Naidu, 2012; Zinla et al., 2021) |
| **Cocoa shell** | 30 | 35 | 10 | 10.91 | 61.17 | 19.78 | 8.14 | 41.59 | 1.67 | 6.18 | 45.98 | 0.10 | (Martínez-Ángel et al., 2015; Tsai et al., 2020) |
| **Cocoa mucilage** | 41.68 | 6.05 | 21.14 | 84.71 | ND | ND | 0.37 | 66.41 | 3.44 | 6.35 | 18.10 | 0.05 | (Widjaja et al., 2021; Saavedra-Sanabria et al., 2021; González Cabra and Suarez Muñoz, 2018) |
| **Corn cob** | 50 | 15.80 | 33.80 | 10.20 | 56.77 | 41.86 | 1.37 | 45.69 | 5.65 | 6.18 | 41.65 | 0.04 | (Montiel and Romero, 2015; Kluska et al., 2020) |
| **Banana peel** | 13 | 14 | 14.80 | 11.56 | 88.02 | 2.70 | 9.28 | 35.65 | 1.94 | 6.19 | 45.94 | 20.75 | (Kumar et al., 2016; Kabenge et al., 2018) |
| **Sugarcane bagasse** | 42.19 | 21.56 | 20.60 | 5.92 | 81.55 | 10.91 | 1.62 | 45.50 | 0.80 | 5.63 | 48.07 | 0.21 | (Álvarez, 2016; Adeniyi et al., 2019; Zamora Rueda et al., 2015) |

Cel: Cellulose, Lig: Lignin, Hemic: Hemicellulose, Hum: Moisture, SV: Volatile solids, CF: Fixed carbon, Cn: Ash, ND: Not determined

The methods of proximal and elemental analysis (Table 3) show potential for reflecting the chemical energy content of biomass, allowing for the evaluation of the sustainability of biorefineries that co-produce bio-oil, biochar, biodiesel, glycerol, and bioelectricity (Aghbashlo et al., 2020). Elemental analysis, or ultimate analysis, estimates the potential emissions of pollutant gases produced during combustion (Rojas et al., 2018). Generally, biomass contains between 70% and 86% volatile matter and a low carbon content, making it a highly reactive fuel. Fuels with low volatile solids can result in flameless combustion, whereas a high volatile solids content can ignite easily (Akowuah et al., 2012; Yang et al., 2017). In bio-oil production, a higher volatile matter content implies a greater yield (Cai et al., 2017). Likewise, the higher the fixed carbon content, the higher the temperature during the energy conversion process, as this is the actual fuel present in the biomass (Palacios Vallejos et al., 2020).

Biomass ash can be used as a fertilizer. However, ash elements can also cause problems during combustion. A high ash content reduces process efficiency, which is why biomass with a low ash content is preferred as a fuel source (Yang et al., 2017; Zając et al., 2018). Determining moisture levels is important because high moisture levels can cause boiler issues during combustion processes, while low moisture levels can lead to accelerated combustion (Ku Ahmad et al., 2018).

Another aspect to consider is the processing method used to transform waste. Biomass is a complex raw material, and its conversion into the final product requires processes that can be classified into four main treatments: physical, chemical, physicochemical, and biological to improve the accessibility of its biopolymers in industrial processing (Orejuela-Escobar et al., 2021; Moreno et al., 2019). Mechanical grinding and extrusion are promising physical pretreatment methods for biomass conversion (Moreno et al., 2019). They are responsible for particle size reduction and increase the surface area of lignocellulosic materials (Kumari and Singh, 2018).

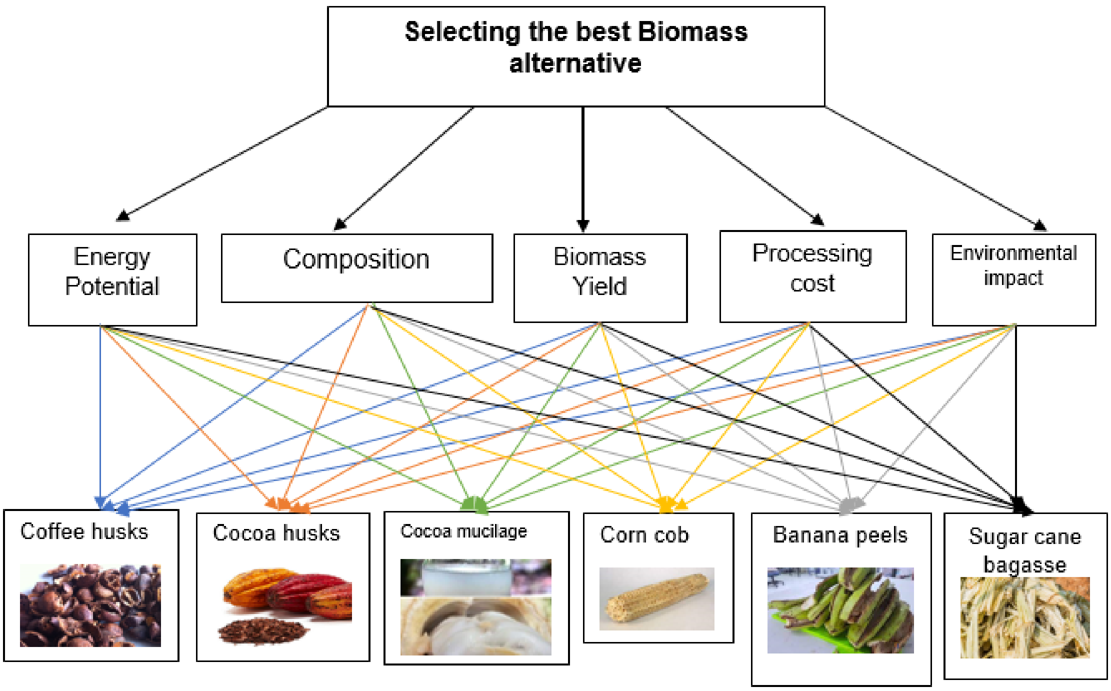


**Figure 1.** Hierarchical structure of the selection of agricultural by-products in the province of Manabí.

Chemical treatment is one of the most promising methods for improving cellulose biodegradability by removing lignin and hemicelluloses (Behera et al., 2014). This treatment can be further classified into alkaline, acidic, sulfite, organosolv, and ionic methods (Oh et al., 2015). In the paper industry, these chemical methods have been used for delignification, which aims to remove non-cellulosic fractions through alkaline treatment (Michelin et al., 2020). Physicochemical conversion includes methods that combine physical changes and chemical reactions during processing, with steam explosion being one of the most widely used physicochemical biomass pretreatments. This type of conversion process leads to the production of high-density biofuels (Jędrzejczyk et al., 2019).

Biological treatments use enzymes or organisms to hydrolyze cellulose and hemicellulose and ferment sugar molecules (Kumari and Singh, 2018). The goal is to produce biofuels, as well as various types of chemicals such as biogas, hydrogen, ethanol, butanol, acetone, etc. Biomass with a high percentage of biodegradable organic matter and high moisture content is generally preferred. The most commonly used processes of this type are anaerobic digestion and fermentation (Zinla et al., 2021; Garba, 2020).

The industrial-scale processing of biomass involves a combination of the described processes, depending on the production system design and the expected products. Considering the above and to select the most important agricultural byproduct for its utilization in a biorefinery facility, the analytical hierarchy process was applied. To achieve this, a multilevel hierarchical structure was developed, linking selection criteria and alternatives (Figure 1). The normalized matrices for each selection criterion were obtained based on the weighting provided by the expert panel (see Annexes). Subsequently, the normalized matrix of the selected criteria (Table 4), the priority of the alternatives (Table 5) and the consistency ratio of each criterion (Table 6) were obtained.

The results obtained indicate that among the evaluated byproducts, the best one for the intended purpose was sugarcane bagasse, with a priority vector of 33.20%, followed by coffee husk at 26.10%. These two biomasses would perform best as raw materials in a small-scale biorefinery within the analyzed context. Regarding the evaluated criteria, composition and environmental impact prevail over the rest, suggesting the possibility of using lignocellulosic residues such as sugarcane.

**Table 4.** Standardized matrix of selected Criteria

| Criterion | PE | C | RB | CP | IA | Normal | Ideal |
|---|---|---|---|---|---|---|---|
| PE | 1 | 0.33 | 0.2 | 1 | 0.33 | 0.074 | 0.221 |
| C | 3 | 1 | 3 | 5 | 1 | 0.334 | 1 |
| RB | 5 | 0.33 | 1 | 5 | 0.33 | 0.202 | 0.606 |
| CP | 1 | 0.2 | 0.2 | 1 | 0.2 | 0.057 | 0.171 |
| IA | 3 | 1 | 3 | 5 | 1 | 0.334 | 1 |

PE: Energy potential, C: Composition, RB: Biomass yield, CP: Cost of processing, IA: Environmental impact

**Table 5.** Priority of alternatives

| Graphic | Alternatives | Total | Normal | Ideal | Classification |
|---|---|---|---|---|---|
| | 1 Coffee husk | 0.131 | 0.261 | 0.787 | 2 |
| | 2 Cacao shell | 0.103 | 0.205 | 0.618 | 3 |
| | 3 Cocoa mucilage | 0.021 | 0.042 | 0.125 | 5 |
| | 4 Corn cob | 0.021 | 0.041 | 0.125 | 6 |
| | 5 Plantain peel | 0.059 | 0.118 | 0.355 | 4 |
| | 6 Sugarcane bagasse | 0.166 | 0.332 | 1 | 1 |


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In a similar study, the sustainability of different biomasses (agricultural and forestry residues) was evaluated using ecological economics tools, including AHP. The results from the multicriteria analysis showed that sugarcane bagasse (Jiménez et al., 2020), with a priority vector of 0.57, had the highest energy contribution. In terms of energy, sugarcane bagasse represents one of the largest sources of bioenergy (Amezcua-Allieri et al., 2019). The results obtained have a consistency index equal to or lower than 0.10, meaning that the consistencies were acceptable and valid for decision-making.

The main reasons for the relatively higher preference for sugarcane bagasse are its richness in polysaccharides, making it a promising raw material for biofuel production and other chemicals under a biorefinery concept. Proper management of this waste resource creates an opportunity to generate additional income (Konde et al., 2021; Restrepo-Serna et al., 2018).

In biotechnological processes, sugarcane bagasse can be used as a carbon source to produce second-generation ethanol, xylitol, biogas, and platform products such as glucose and xylose, from which other high-value compounds can be derived (Antunes et al., 2021; Nosratpour et al., 2018). In recent years, succinic acid, a value-added chemical, has been derived from sugarcane bagasse and investigated as a biorefinery co-product (Nieder-Heitmann et al., 2019). Additionally, this residual biomass can be used in fermentation processes to obtain compounds such as butanol, lactic acid, and poly-3-hydroxybutyrate (PHB), which have been identified for its inclusion in the range of multiproduct biorefineries (Restrepo-Serna et al., 2018).

Second in priority is coffee husk, whose interest arises due to its high potential value. Given its composition rich in polysaccharides, along with a significant number of other active biomolecules, it is possible to obtain value-added products from this biomass (Oliveira et al., 2021; Mora-Villalobos et al., 2021).

Coffee husk can yield bioproducts such as citric acid, lactic acid, polyhydroxyalkanoates, biofuels, cosmetics, and others (Aristizábal-Marulanda et al., 2017; Iriondo-DeHond et al., 2020). Additionally, coffee husk has been proposed as a filler in polymeric matrices as a low-cost alternative. Moreover, due to its high cellulose and hemicellulose content, along with its high calorific value, it is a promising raw material for bioenergy production (Rambo et al., 2015; Sisti et al., 2021).

**Table 6.** Consistency ratio obtained in the criteria.

| Matrix | RC |
|---|---|
| Energy Potential | 0.103 |
| Composition | 0.096 |
| Biomass Yield | 0.099 |
| Processing Cost | 0.088 |
| Environmental Impact | 0.093 |

# 4 Conclusions

The application of an analytical hierarchy process allowed for the identification of the best biomass option to be used as a raw material in a small-scale biorefinery in the province of Manabí. The most important evaluation criteria were raw material composition and environmental impact, both of which received equal global weight among all those assessed. Through multicriteria analysis, it was determined that sugarcane bagasse is the most promising alternative compared to the other studied biomasses.

Thus, this agricultural residue represents an interesting alternative for the country's bioeconomic progress, as it creates opportunities for the development of new products and participation in the global market. Lignocellulosic residues are renewable sources, and their composition and structural properties have significant effects on their conversion within a biorefinery processing perspective. Understanding the composition of these residues makes it possible to predict the type of treatment required to obtain a wide range of bioproducts.

# Authors' contribution

J.M.S.B.: Research, methodology, writing- original draft, visualization. G.A.Z.V.: Research, methodology, writing- original draft, visualization. R.E.C.C.: Validation. M.A.R.: Conceptualization, supervision, validation, writing- review and editing.

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# Appendix

**Table A 1.** Normalized energy potential criteria matrix.

| | Coffee husk | Cocoa shell | Cocoa mucilage | Corn cob | Banana peel | Sugarcane bagasse | Normal | Ideal |
|---|---|---|---|---|---|---|---|---|
| **Coffee husk** | 1 | 5 | 5 | 5 | 5 | 0.2 | 0.246 | 0.495 |
| **Cocoa shell** | 0.2 | 1 | 1 | 0.333 | 0.2 | 0.143 | 0.036 | 0.073 |
| **Cocoa mucilage** | 0.2 | 1 | 1 | 0.333 | 0.333 | 0.143 | 0.039 | 0.078 |
| **Corn cob** | 0.2 | 3 | 3 | 1 | 0.333 | 0.143 | 0.068 | 0.137 |
| **Banana peel** | 0.2 | 5 | 3 | 3 | 1 | 0.2 | 0.115 | 0.231 |
| **Sugarcane bagasse** | 5 | 7 | 7 | 7 | 5 | 1 | 0.497 | 1 |

**Table A 2.** Normalized composition criteria matrix

| | Coffee husk | Cocoa shell | Cocoa mucilage | Corn cob | Banana peel | Sugarcane bagasse | Normal | Ideal |
|---|---|---|---|---|---|---|---|---|
| **Coffee husk** | 1 | 0.333 | 7 | 7 | 5 | 1 | 0.231 | 0.530 |
| **Cocoa shell** | 3 | 1 | 7 | 5 | 5 | 5 | 0.435 | 1 |
| **Cocoa mucilage** | 0.143 | 0.143 | 1 | 1 | 0.333 | 0.143 | 0.035 | 0.079 |
| **Corn cob** | 0.143 | 0.2 | 1 | 1 | 1 | 0.143 | 0.046 | 0.107 |
| **Banana peel** | 0.2 | 0.2 | 3 | 1 | 1 | 1 | 0.081 | 0.187 |
| **Sugarcane bagasse** | 1 | 0.2 | 7 | 7 | 1 | 1 | 0.172 | 0.397 |

**Table A 3.** Normalized biomass yield criteria matrix

| | Coffee husk | Cocoa shell | Cocoa mucilage | Corn cob | Banana peel | Sugarcane bagasse | Normal | Ideal |
|---|---|---|---|---|---|---|---|---|
| **Coffee husk** | 1 | 5 | 7 | 7 | 3 | 0.2 | 0.234 | 0.471 |
| **Cocoa shell** | 0.2 | 1 | 3 | 5 | 0.333 | 0.143 | 0.075 | 0.150 |
| **Cocoa mucilage** | 0.143 | 0.333 | 1 | 1 | 0.2 | 0.143 | 0.033 | 0.067 |
| **Corn cob** | 0.143 | 0.2 | 1 | 1 | 0.2 | 0.143 | 0.032 | 0.064 |
| **Banana peel** | 0.333 | 3 | 5 | 5 | 1 | 0.2 | 0.129 | 0.260 |
| **Sugarcane bagasse** | 5 | 7 | 7 | 7 | 5 | 1 | 0.497 | 1 |

**Table A 4.** Normalized processing cost criteria matrix

| | Coffee husk | Cocoa shell | Cocoa mucilage | Corn cob | Banana peel | Sugarcane bagasse | Normal | Ideal |
|---|---|---|---|---|---|---|---|---|
| **Coffee husk** | 1 | 1 | 1 | 5 | 1 | 0.333 | 0.148 | 0.401 |
| **Cocoa shell** | 1 | 1 | 3 | 9 | 7 | 3 | 0.370 | 1 |
| **Cocoa mucilage** | 1 | 0.333 | 1 | 3 | 3 | 0.333 | 0.117 | 0.317 |
| **Corn cob** | 0.2 | 0.111 | 0.333 | 1 | 1 | 0.143 | 0.037 | 0.101 |
| **Banana peel** | 1 | 0.143 | 0.333 | 1 | 1 | 0.2 | 0.060 | 0.161 |
| **Sugarcane bagasse** | 3 | 0.333 | 3 | 7 | 5 | 1 | 0.267 | 0.721 |

**Table A 5.** Normalized environmental impact criteria matrix

| | Coffee husk | Cocoa shell | Cocoa mucilage | Corn cob | Banana peel | Sugarcane bagasse | Normal | Ideal |
|---|---|---|---|---|---|---|---|---|
| **Coffee husk** | 1 | 7 | 9 | 5 | 3 | 1 | 0.328 | 0.875 |
| **Cocoa shell** | 0.143 | 1 | 1 | 5 | 0.2 | 0.143 | 0.063 | 0.169 |
| **Cocoa mucilage** | 0.111 | 1 | 1 | 1 | 0.2 | 0.143 | 0.040 | 0.106 |
| **Corn cob** | 0.2 | 0.2 | 1 | 1 | 0.2 | 0.143 | 0.036 | 0.097 |
| **Banana peel** | 0.333 | 5 | 5 | 5 | 1 | 0.2 | 0.158 | 0.421 |
| **Sugarcane bagasse** | 1 | 7 | 7 | 7 | 5 | 1 | 0.375 | 1 |

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# OBTAINING BIOETHANOL FROM COCOA SHELLS (*THEOBROMA CACAO*) USING *TRICHODERMA REESEI* AND *TRICHODERMA GHANENSE* FOR ENZYMATIC HYDROLYSIS

## OBTENCIÓN DE BIOETANOL A PARTIR DE LA CÁSCARA DE CACAO (*THEOBROMA CACAO*) USANDO *TRICHODERMA REESEI* Y *TRICHODERMA GHANENSE* PARA LA HIDRÓLISIS ENZIMÁTICA

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**Abstract**

The use of fossil fuels generates Greenhouse Gases (GHG), one of the main causes of global overheating, which has become a problem in recent decades. The use of second generation of biofuels has been perceived as an alternative to replace or reduce the use of fossil fuels; for this reason, the present work aims to obtain bioethanol from cocoa shell (*Theobroma cacao*) of the clone CCN-51 obtained in Los Rios Province, Ecuador, through a series of steps involving: a) alkaline pretreatment, b) enzymatic hydrolysis using two species of endophytic fungi from the same cocoa shell (*Trichoderma reesei* and *Trichoderma ghanense*) at different concentration and c) alcoholic fermentation using *Saccharomyces cerevisiae* yeast. The amount of bioethanol obtained from the process was determined by gas chromatograph with a flame ionization detector (FID). The results show a moderate production of bioethanol ranging from 0.024% v/v to

0.254 % v/v, which indicates that the cocoa shell (*Theobroma cacao*) of clone CCN-51 is a potential matrix to bioethanol production.

***Keywords***: *Theobroma cacao*, *Trichoderma*, biomass, bioethanol, alcoholic fermentation.

**Resumen**

El uso de combustibles fósiles genera gases de efecto invernadero (GEI), uno de los principales causantes del sobrecalentamiento global, problemática de gran interés en las últimas décadas. El uso de biocombustibles de segunda generación se ha vislumbrado como alternativa para sustituir o disminuir el uso de combustibles fósiles. Por esta razón, el presente trabajo tiene como objetivo obtener bioetanol a partir de la cáscara de cacao (*Theobroma cacao*) del clon CCN-51 obtenido en la Provincia de Los Ríos, Ecuador, por medio de una serie de pasos que involucran: a) pretratamiento alcalino, b) hidrólisis enzimática usando dos especies de hongos endófitos de la misma cáscara de cacao (*Trichoderma reesei* y *Trichoderma ghanense*) a diferentes concentraciones y c) fermentación alcohólica usando levadura *Saccharomyces cerevisiae*. La cantidad de bioetanol obtenida del proceso fue determinada por medio de un cromatógrafo de gases con detector de ionización de llama (FID). Los resultados muestran una producción moderada de bioetanol que va desde 0,024 % v/v a 0,254 % v/v lo que indica que la cáscara de cacao (*Theobroma cacao*) del clon CCN-51 es una matriz potencial para la producción de bioetanol.

***Palabras clave***: *Theobroma cacao*, *Trichoderma*, biomasa, bioetanol, fermentación alcohólica.

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# 1 Introduction

Over time, human activity and industrial development have positioned fossil fuels as the primary energy source. Throughout their extraction and production processes, a significant number of countries, including Ecuador, have experienced economic benefits. However, these processes have also triggered various problems that extend beyond the producing countries and impact the global community. One notable example is the emission of greenhouse gases (GHGs), which are largely responsible for global warming.

In recent decades, scientific research has increasingly focused on mitigating these environmental impacts, leading to a growing interest in second-generation biofuels (Wahono et al., 2014; Oliva et al., 2017). This interest is largely due to the use of lignocellulosic biomass in their production-biomass that can be sourced from a variety of materials, such as agro-industrial residues from sugarcane and maize cultivation (Antizar-Ladislao and Turrion-Gomez, 2008). This enables the valorization of plant material that is typically discarded (Khan et al., 2025), resulting in a dual environmental benefit: first, the avoidance of waste accumulation on land surfaces and the need for its disposal through incomplete combustion, which is even more polluting (Cury R et al., 2017; Orejuela-Escobar et al., 2021); and second, the use of these second-generation fuels significantly reduces the proportion of GHGs released into the environment (Morais et al., 2020).

Preliminary studies suggest that residues from agricultural crops (such as stalks, leaves, and husks), non-food crops, forest residues, and agro-industrial waste could potentially support the required bioethanol supply (Anwar et al., 2014). Ecuador, as a country rich in plant diversity, has the capacity to generate lignocellulosic material suitable for this purpose.

According to records from 2007 to 2012, Ecuador experienced a 184 % increase in the export of roasted cocoa beans and cocoa husks, positioning the country as one of the world's leading cocoa producers and exporters, reaching the fourth place globally (Teneda Llerena et al., 2019). Within the chocolate industry (Caviedes Rubio et al., 2024), only the cocoa beans-constituting approximately 30 % of the entire fruit-are utilized in production, while the remaining 70 % (husks and mucilaginous pulp) is discarded (Sarmiento Hernández, 2019). Considering the substantial amount of waste generated annually in Ecuador from this plant species, it has been estimated that bioethanol production from such biomass could serve as an alternative pathway for energy generation, significantly contributing to national fuel consumption (Sigüencia Avila et al., 2020). However, this potential has yet to be realized.

Moreover, it is important to note that the composition of lignocellulosic material varies depending on its origin (Anwar et al., 2014), and cocoa husks are no exception. They are reported to have a low cellulose content that fluctuates depending on the type of cocoa, along with a high proportion of lignin and hemicellulose-components that interfere with the conversion of cellulose into biofuel (Sarmiento Hernández, 2019). Combined with a general lack of information and awareness on the subject, these factors constitute the primary reasons why agro-industrial residues are not being properly utilized.

The chemical and enzymatic reactions necessary for bioethanol production are directly affected by the composition of lignocellulosic material (Winarsih and Siskawardani, 2020). In light of this and the challenges mentioned above, it is essential to develop alternative approaches that enable the use of biomass with conversion rates that make the process economically viable.

Accordingly, lignocellulosic material must undergo pretreatment followed by enzymatic hydrolysis-a method proven to be effective, cost-efficient, and specific for obtaining fermentable sugars under mild reaction conditions (Winarsih and Siskawardani, 2020). The efficiency of this process depends on several factors including pH, fermentation time, substrate type (biomass), temperature, and enzymatic activity, among others (Anwar et al., 2014). Numerous microorganisms are capable of degrading cellulose, among which fungi of the genus *Trichoderma* are the most common (Nasir Iqbal et al., 2011; Rosyida et al., 2015). In particular, the species *Trichoderma reesei* is the most commercially used due to its widespread industrial application in the saccharification of cellulose into simple sugars for biofuel production (Adav et al., 2012; Peciulyte et al., 2014).

This study aims to explore the necessary conditions for producing bioethanol from cocoa biomass, taking into account the complications associated with working with this particular substrate. The objective is to propose viable alternatives that enhance the accessibility and efficiency of the process, thereby promoting the effective use of these agro-industrial residues.

# 2 Materials and Methods

## 2.1 Sample collection and processing

### 2.1.1 Sample collection

The cocoa husks (*Theobroma cacao*) from the CCN-51 clone were collected in April 2020 from a private estate located in the Buena Fe district, Los Ríos Province, Ecuador.

### 2.1.2 Sample Treatment

**Drying and Milling**

The cocoa husks were cut into pieces of approximately 1 $cm^3$ and air-dried for 7 days. Subsequently, they were manually ground using a low-hopper Corona plate mill. The resulting particles were sieved using a USA Standard Test Sieve mesh #18 with 1 mm porosity. Particles smaller than 1 mm were separated and stored in a desiccator.

**Removal of Volatile Extractives**

This process followed the NREL/TP-510-42619 reference method (Sluiter et al., 2005), in which 10.0000 g of the sieved sample were subjected to a two-stage Soxhlet extraction: first with 200 mL of distilled water for 2 hours, and then with 200 mL of ethanol for an additional 2 hours.

## 2.2 Biomass Characterization

Characterization was conducted following procedures from AOAC International, ASTM International, NREL, and TAPPI. All analyses were performed in triplicate.

### 2.2.1 Moisture Content Determination

Moisture content was determined in accordance with AOAC 934.01 (AOAC, 2012). A 1.0000 g sample was weighed into a crucible, placed in a preheated oven at 105 °C, and dried for 3 hours. After drying, the crucible was transferred to a desiccator until it reached room temperature, then weighed. The drying process was repeated in 1-hour intervals until a constant weight was achieved.

### 2.2.2 Ash Content Determination

Ash content was measured following AOAC 942.05 (Thiex et al., 2012), using incineration at 550 °C. The crucible containing the previously dried sample was placed in a preheated muffle furnace for 5 hours. After cooling in a desiccator to room temperature, it was weighed and recorded. The process was repeated for 1-hour intervals until constant weight was obtained.

### 2.2.3 Holocellulose Content Determination

A 4.0000 g sample was placed in an Erlenmeyer flask and treated with 300 mL of distilled water, 0.4 mL of glacial acetic acid, and 2.0000 g of sodium chlorite. The flask was heated in a water bath (Memmert) at 75 °C for 1 hour. This process was repeated three times until a whitish coloration was observed. The mixture was then cooled in an ice bath at 10 °C, centrifuged at 3500 rpm for 15 minutes, and vacuum-filtered. The filtered and washed product was dried in an oven at 105 °C for 4 hours, then transferred to a desiccator until room temperature was reached, and weighed. The drying cycle was repeated until a constant weight was reached. Final holocellulose content was determined by the difference in weight between the treated crucible and the empty dried crucible (Nomanbhay et al., 2013).

### 2.2.4 Cellulose Content Determination

Cellulose content was determined according to ASTM D16-96-95(2019)e1 (ASTM International, 2019). A 2.0000 g holocellulose sample was treated in an Erlenmeyer flask with 10 mL of 17.5% sodium hydroxide (rested for 5 minutes), followed by an additional 5 mL (rested for 30 minutes), and then 30 mL of distilled water (rested for 1 hour). The mixture was vacuum filtered, washed three times with water-sodium hydroxide solution, followed by 30 mL of water. Then, 5 mL of 10 % acetic acid and 50 mL of distilled water were added, and the sample was vacuum filtered again. The residue was dried in an oven at 105 °C for 12 hours, transferred to a desiccator to cool, and weighed until constant mass was reached.

#### 2.2.5 Hemicellulose Content Determination

Hemicellulose content was calculated as the difference between the holocellulose and cellulose content, following Loja Sánchez (2016).

#### 2.2.6 Lignin Content Determination

Lignin content was determined following TAPPI T-222 om-02 (TAPPI, 2002), which assesses acid-insoluble lignin in wood and unbleached pulp. Approximately 1 g of dried cocoa husk sample was placed in a flask with 15 mL of 72 % sulfuric acid and stirred at 400 rpm for 1 hour using a ColeParmer mechanical shaker. The sample was transferred to a 250 mL flask with 125 mL distilled water and refluxed for 4 hours. It was then vacuum filtered, washed with 500 mL hot water, dried at 105 °C for 3 hours, cooled in a desiccator, weighed, and re-dried to constant weight.

### 2.3 Bioethanol Production

#### 2.3.1 Alkaline Pretreatment

Following Jannah and Asip (2015), the milled cocoa husk biomass was treated with 3 % sodium hydroxide until reaching a pH of 11. The lignocellulosic biomass was immersed in the solution at a solid/liquid ratio of 1:10 (100 g sample/1000 mL of 3 % NaOH), incubated at 121 °C for 90 minutes to enhance biomass swelling and enzymatic accessibility. The sample was filtered and neutralized with distilled water washes and 30 % HCl until the filtrate reached pH 5. The biomass was then dried at 60 °C for 24 hours.

#### 2.3.2 Enzymatic Hydrolysis Using Endophytic Fungi *Trichoderma reesei* and *Trichoderma ghanense*

The method, based on NREL TP-510.42629 (Selig et al., 2008), involved cellulase-mediated hydrolysis of cellulose into low-molecular-weight sugars. Enzyme-producing endophytic fungi (*Trichoderma reesei* and T. ghanense) were obtained from the microbial culture collection of the CIBE, Escuela Superior Politécnica del Litoral (ESPOL). Preparation steps included:

- **Re-inoculation:** Three replicates were grown on PDA plates at 26 °C for 7 days until sporulation.
- **Spore Washing:** Spores were removed using 10 mL of saline solution with mechanical agitation.
- **Spore Suspension:** Concentrations were adjusted to $1\times10^7$ and $1\times10^9$ spores/mL using a Neubauer chamber.
- **Substrate Preparation and Inoculation:** 50 g of cocoa husk substrate per sample were sterilized at 120 °C for 15 minutes and dried at 60 °C for 24 hours. The moisture content was adjusted to 75 % with 35 mL of sterile distilled water. Then, 10 mL of the fungal spore solution (at both concentrations) was added to each flask. Samples were incubated at 25 °C for 10 days.

#### 2.3.3 Alcoholic Fermentation

*Saccharomyces cerevisiae* yeast was used due to its high fermentative capacity (Van Zyl et al., 2007). Yeast activation involved mixing 0.5 g sugar and 15 g yeast in 75 mL of sterilized distilled water at 28 °C for 20 minutes. The volume was adjusted to 250 mL and added to bioreactors under five scenarios:

- **Scenario 1:** 50 g of alkaline pretreated cocoa husk.
- **Scenarios 2 and 3:** Alkaline pretreated and enzymatically hydrolyzed biomass with T. reesei at $1\times10^7$ and $1\times10^9$ spores/mL, respectively.
- **Scenarios 4 and 5:** Same as above but with T. ghanense at $1\times10^7$ and $1\times10^9$ spores/mL

Fermentation was anaerobic, with bioreactors sealed and $CO_2$ released through a purge hose submerged in distilled water. The process lasted 4 days at ambient temperature (∼25 °C) in darkness. After fermentation, the liquid was filtered, allowed to settle in test tubes, and the supernatant was decanted for analysis.

### 2.4 Ethanol Quantification

Ethanol was quantified using a Thermo Scientific gas chromatograph equipped with a flame ionization detector. The ethanol concentration was expressed in mg% AA and converted to mL of ethanol per gram of cocoa husk biomass. A calibration curve using absolute ethanol was prepared to determine ethanol concentration in the samples (Mansur et al., 2022). Analytical conditions were optimized, as summarized in Table 1.

**Table 1.** Chromatographic conditions

| | |
|---|---|
| Equipment | Thermo Fisher gas chromatograph (GC), TRACE GC 1300 Series |
| Carrier gas | Helium |
| Flow rate | 2 mL/min |
| Injection volume | 1 uL |
| Injection mode | Split |
| Injector temperature | 180 °C |
| Column | J&W Scientific DB-FFAP, 60 m × 0.250 mm (ID) × 0.25 $\mu$m |
| Stationary phase | Modified polyethylene glycol acid |
| Oven temperature | 180 °C |
| Detector | Flame Ionization Detector (FID) |
| Detector temperature | 250 °C |

## 3 Results and Discussion

### 3.1 Biomass Characterization

The analysis of cocoa husk (*Theobroma cacao*) from the CCN-51 clone was carried out to determine the most relevant parameters for ethanol production, namely: moisture, ash, holocellulose, cellulose, hemicellulose, and lignin. These parameters were used to establish the optimal conditions for pretreatment, enzymatic hydrolysis, and the fermentation process. Their values guided the selection of the most effective procedures to ensure proper ethanolic fermentation.

The moisture content obtained was 11.16%, as shown in Table 2. This value is higher than that reported by Vivanco Carpio et al. (2018), who presented average values of 8.74% for national cocoa and 6.43% for CCN-51 cocoa collected from the province of El Oro. This difference may be attributed to the specific climatic conditions of the mountainous cultivation area in the Buena Fe canton, Los Ríos Province, where the humus-rich soil is suitable for various crops and to the environmental humidity exposure during the transport of the cocoa husk to the laboratory.

The average ash content in the evaluated lignocellulosic biomass was 10.70%, a value comparable to that reported by Villamizar Jaimes et al. (2021) for Colombian cocoa husks of the same variety, with estimated values of 10.77% and 11.39%, depending on whether the sample was untreated or oven-dried. In contrast, Vivanco Carpio et al. (2018) reported an average ash content of 5.54% for Ecuadorian CCN-51 cocoa husks, and 5.14% for national cocoa. Similarly, Castillo et al. (2018) reported 8.59% for this variety of Venezuelan cocoa.

The variation in ash content observed in different studies may be influenced by climatic and edaphic factors. This was demonstrated by Chafla et al. (2016) in a study conducted in various Amazonian provinces on CCN-51 cocoa husks, where it was shown that soil quality, mineral composition, and moisture saturation significantly affect the ash content. Therefore, it can be inferred that the current sample was influenced by the mineral composition of the mountainous soil.

According to the results in Table 2, the average cellulose and hemicellulose contents were 26.08% and 5.38%, respectively, both with a variation coefficient below 2%. Torres (2016) reported similar results, with an average cellulose content of 24.02% for the same matrix, while hemicellulose content averaged 2.23%, though with a high variability (31.44%), indicating inconsistency in results. In that case, the variation in hemicellulose content was attributed to the use of pH adjustment with sulfuric acid, an inorganic compound that may degrade this polysaccharide (Torres, 2016). Likewise, Loayza (2020) confirmed cellulose and hemicellulose contents of 29.09% and 2.97%, respectively, using acid treatment of the biomass.

**Table 2.** Moisture, ash, holocellulose, cellulose, hemicellulose and lignin content of cocoa shells.

| **Parameter** | $\bar{X}$ | σ | $C_v$ |
|---|---|---|---|
| Moisture | 11.16 % | 0.05 | 0.54 % |
| Ash | 10.70 % | 0.34 | 2.71 % |
| Holocellulose | 31.80 % | 0.25 | 0.79 % |
| Cellulose | 26.24 | 0.25 | 0.09 % |
| Hemicellulose | 5.38 | 0.09 | 1.66 % |
| Lignin | 28.52 % | 0.75 | 2.62 % |

The total lignin content of biomass includes both acid-insoluble lignin (AIL) and, to a lesser extent, acid-soluble lignin (ASL), with the former being more easily identified due to its abundance in lignocellulosic biomass and the use of gravimetric methods. Literature confirms that lignin is the most abundant compound in cocoa biomass, as supported by Encalada and Jácome (2018), who reported an average content of 25.81 %, and Vásquez (2010), who found concentrations ranging from 14.6 % to 26.38 %. Other studies report even higher total lignin contents, exceeding 40 %, such as Benalcázar (2018) with 46.61 %, and Torres (2016), who reported values ranging from 33.43 % to 45.39 % based on multiple analyses. This study focused on the determination of acid-insoluble lignin, as it is the predominant polymer in the biomass of interest, while the soluble fraction is typically lost during the extraction of volatile compounds. The average value obtained was 28.52 %, as shown in Table 2, with a variation coefficient of 2.62 %, confirming the precision of the results.

## 3.2 Bioethanol Production and Quantification

During alkaline pretreatment of the biomass using 3 % sodium hydroxide, physical changes were observed as a result of lignin removal. One noticeable change was biomass swelling and a color transformation from the characteristic brown of dry cocoa husk to black after pretreatment. This was attributed to the breakdown of ester bonds in the plant material and the elimination of reddish lignin. After pH adjustment, the biomass underwent another color change, turning from black to light brown.

Enzymatic hydrolysis was conducted using two different fungi: *Trichoderma reesei* and *Trichoderma ghanense*, with spore concentrations of $1\times10^7$ and $1\times10^9$ spores/mL for both species. After 10 days of incubation at room temperature in contact with the biomass inside the bioreactors, greenish coloration was observed on the surface of the biomass, with more prominent growth along the reactor walls-an indication of fungal development. Additionally, biomass swelling was observed, consistent with the conversion of cellulose and hemicellulose into fermentable sugars.

Following enzymatic hydrolysis, alcoholic fermentation was carried out using *Saccharomyces cerevisiae* yeast for 4 days. During this period, intense bubbling was observed at the outlet of the bioreactor, indicating $CO_2$ emission as a byproduct of alcoholic fermentation. Biomass swelling and abundant yeast growth on the reactor surface were also noted. Upon opening the reactor, a strong odor characteristic of fermentation processes was detected.

Before analyzing the fermented products, a calibration curve was prepared using five ethanol standard concentrations: 0.1, 0.3, 0.5, 0.7, and 0.9 % (v/v). The calibration curve yielded a satisfactory correlation coefficient (R = 0.9931). Interpretation of the resulting chromatograms was based on the ethanol elution peak, using retention time and peak area data.

Analysis of the samples showed that Scenario 1, which did not include enzymatic hydrolysis, resulted in the highest ethanol yield (0.25 % v/v), in contrast to Scenario 2 (less than 0.1 % v/v), Scenario 3 (below 0.15 % v/v), Scenario 4 (under 0.22 % v/v), and Scenario 5 (below 0.15 % v/v), as shown in Table 3.

According to the values obtained in Scenario 1, the results are comparable to those reported by Benalcázar (2018), who achieved an ethanol concentration of 0.57 % v/v using alkaline hydrolysis treatment under conditions similar to those in the present study. This confirms that the method is effective for cocoa husk due to its ability to break the bonds linking lignin and hemicellulose chains. However, the yield from biological treatment remains relatively low.

**Table 3.** Bioethanol production results

| Scenario | Sample | Retention Time (min) | Ethanol % v/v |
|---|---|---|---|
| Scenario 1: Alkaline Pretreatment | Blank 1 | 4.187 | 0.267 |
| | Blank 2 | 4.183 | 0.133 |
| | Blank 3 | 4.190 | 0.362 |
| | | | $\bar{X}$ = 0.254 |
| Scenario 2: Alkaline Pretreatment + *T. reesei* $1\times10^7$ spores/mL | E2M1 | 4.185 | 0.083 |
| | E2M2 | 4.183 | 0.145 |
| | E2M3 | 4.183 | 0.026 |
| | | | $\bar{X}$ = 0.084 |
| Scenario 3: Alkaline Pretreatment + *T. reesei* $1\times10^9$ spores/mL | E3M1 | 4.185 | 0.029 |
| | E3M2 | 4.183 | 0.045 |
| | E3M3 | 4.183 | 0.000 |
| | | | $\bar{X}$ = 0.024 |
| Scenario 4: Alkaline Pretreatment + *T. ghanense* $1\times10^7$ spores/mL | E4M1 | 4.183 | 0.1256 |
| | E4M2 | 4.185 | 0.2251 |
| | E4M3 | 4.183 | 0.0559 |
| | | | $\bar{X}$ = 0.1355 |
| Scenario 5: Alkaline Pretreatment + *T. ghanense* $1\times10^9$ spores/mL | E5M1 | 4.185 | 0.1444 |
| | E5M2 | 4.185 | 0.1057 |
| | E5M3 | 4.187 | 0.0686 |
| | | | $\bar{X}$ = 0.1062 |

The composition of lignocellulosic biomass varies depending on the substrate type. Thus, the levels of cellulose and hemicellulose present determine the efficiency of sugar-to-ethanol conversion. This is supported by the findings of Casabar et al. (2019) in their study on bioethanol production from pineapple peels, which showed that a decrease in reducing sugar corresponds with an increase in ethanol production. This is primarily due to the fermentable sugars derived from cellulose and hemicellulose hydrolysis, which are utilized by *Saccharomyces cerevisiae* during fermentation to produce ethanol.

In this study, the cocoa husk matrix contained 26.24% cellulose and only 5.38% hemicellulose, with the latter potentially limiting the generation of reducing sugars. The availability of hemicellulose for fermentation may have been negatively affected by the applied hydrolysis pretreatment, which disrupts the structural bonds between lignin and carbohydrates, leading to hemicellulose degradation and lignin solubilization. This progressively reduces polymer content for conversion into sugars, as also observed by Sánchez Riaño et al. (2010) in various chemical pretreatment methods for biomass.

Based on these findings, Scenario 1 achieved the highest bioethanol yield under the tested conditions. Nevertheless, the yield was limited by two key factors: the type of pretreatment, as previously described, and the lignin content in the substrate, as this polymer restricts cellulose and hemicellulose hydrolysis (Ko et al., 2015). Literature suggests that enzymatic hydrolysis using *Trichoderma* spp. could enhance the production of reducing sugars via cellulase enzymes. For instance, López et al. (2014) demonstrated that banana peel biomass yielded up to 5.18 % v/v ethanol using *Trichoderma* species, attributed to its 23 % cellulose and 23 % hemicellulose content.

However, in this study, scenarios treated with *Trichoderma reesei* produced low ethanol yields (0.084 % v/v and 0.024 % v/v), while *Trichoderma ghanense* yielded 0.1355 % v/v and 0.1062 % v/v, respectively. These results indicate that biomass composition significantly affects fungal enzymatic performance, particularly due to the limited hemicellulose content. Additionally, the low yields in Scenarios 2 to 5 suggest that other factors, such as the hydrolysis duration and fungal concentration, also influence outcomes. These two variables interact-once cellulose degradation is complete, the fungus may consume the resulting glucose for survival.

Overall, the factors that most significantly impacted ethanol production were biomass composition, pretreatment method, and fungal concentration. In this context, the findings support a negative hypothesis regarding the effectiveness of fungal application on cocoa lignocellulosic residues for ethanol production, due to the low yields obtained with phytopathogenic fungi.

Furthermore, comparing ethanol yields between the scenarios involving *Trichoderma reesei* and *Trichoderma ghanense*, the latter exhibited higher ethanol production.

# 4 Conclusions

This study provided a well-characterized lignocellulosic matrix with high lignin content and low hemicellulose content, in alignment with previously reported data for the same cocoa husk variety. As such, cocoa husk presents itself as a viable substrate for biomass-to-ethanol conversion processes.

Based on the variables tested during this comparative fermentation study, the optimal conditions for ethanol production were achieved using alkaline pretreatment, with 50 g of biomass incubated at room temperature over a period of 3 days. Scenario 1 yielded the highest ethanol concentration at 0.25 % v/v. However, for cocoa husk to be more efficient as a bioethanol feedstock, a higher hemicellulose content is necessary. In combination with cellulose, this would enable the generation of sufficient reducing sugars to significantly enhance ethanol yields through enzymatic hydrolysis with *Trichoderma reesei* and *Trichoderma ghanense*.

# Author Contributions

V.P.J.E.: Conceptualization, methodology, resources, supervision, review, and writing-editing. T.Z.Z.: Conceptualization, methodology, project administration, visualization, and original draft writing. L.L.G.R.: Methodology and resources. M.M.M.: Methodology, supervision, validation, review, and writing-editing. J.V.V.: Investigation and resources. V.A.C.M.: Investigation, formal analysis, and validation. F.C.C.C.: Investigation, formal analysis, and validation. D.B.C.: Investigation, formal analysis, and validation. L.V.M.: Investigation, formal analysis, and validation. R.F.E.L.: Investigation and resources.

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