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CONSORCIOS BACTERIANOS PARA LA INOCULACIÓN Y RESTABLECIMIENTO DE LA ESPECIE EXTINTA EN RAPA NUI: *SOPHORA TOROMIRO*

Tesis para optar al grado de Magister en Ciencias Agronómicas

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RESUMEN

Sophora toromiro (Phil.) Skottsb. es un arbusto leguminoso endémico de la isla de Rapa Nui, Chile, actualmente extinto en su hábitat natural. Sus intentos de reintroducción se han visto obstaculizados por suelos degradados, escasez hídrica, y la ausencia de bacterias fijadoras de nitrógeno compatibles. Este estudio evaluó el potencial de consorcios microbianos compuestos por *Mesorhizobium* sp. y rizobacterias promotoras del crecimiento vegetal (PGPR, por sus siglas en inglés) de mejorar la nodulación y la tolerancia a la sequía en *S. toromiro* propagado por estacas. Las cepas bacterianas fueron aisladas de suelos asociados a especies del género *Sophora* en distintas zonas de Chile, incluyendo Rapa Nui e Isla Magdalena, y fueron caracterizadas y evaluadas por su compatibilidad. Los ensayos se realizaron en invernadero bajo condiciones controladas, incluyendo un periodo de sequía de dos semanas para simular las limitaciones hídricas estacionales de la isla. Los tratamientos inoculados mejoraron la nodulación y mostraron tendencias favorables en el desarrollo radicular en comparación con los controles no inoculados. Si bien no se observaron diferencias estadísticamente significativas en otros parámetros morfológicos (altura del brote principal, crecimiento del brote principal, peso seco aéreo, peso seco radicular y biomasa total) algunas plantas co-inoculadas mantuvieron su biomasa radicular relativa bajo sequía, lo que sugiere un posible efecto benéfico de la interacción microbiana. El uso de material vegetal propagado vegetativamente y la corta duración del ensayo podrían haber influido en los resultados, lo que resalta la necesidad de considerar estas variables en estudios futuros, especialmente en especies de crecimiento lento. En conjunto, la selección y aplicación de consorcios bacterianos nativos y compatibles mostró un potencial prometedor para apoyar la restauración ecológica de *S. toromiro* en Rapa Nui.

SUMMARY

Sophora toromiro (Phil.) Skottsb. is a leguminous shrub endemic to Rapa Nui Island, Chile, currently extinct in its natural habitat. Its reintroduction is challenged by degraded soils, water scarcity, and the absence of compatible nitrogen-fixing bacteria. This study evaluated the potential of microbial consortia, composed of *Mesorhizobium* sp. and plant growth-promoting rhizobacteria (PGPR), to improve nodulation and drought tolerance in *S. toromiro* propagated by stem cuttings. Bacterial strains were isolated from soils associated with *Sophora* species across Chile, including Rapa Nui and Magdalena Island, and were characterized and tested for compatibility. Greenhouse assays were conducted under controlled conditions, including a two-week drought period to simulate the island's seasonal water limitations. Inoculated treatments enhanced nodulation and showed favorable trends in root development compared to uninoculated controls. Although no statistically significant differences were observed in other morphological parameters (main shoot height, main shoot growth, aerial dry weight, root dry weight and total biomass), these trends suggest a beneficial effect of co-inoculation, since some co-inoculated plants maintained the same relative root biomass under dry conditions. The use of vegetatively propagated plant material and the relatively short trial duration may have influenced the outcomes, highlighting the need to consider these variables in future studies, especially in slow-growing species. Overall, the selection and application of compatible native bacterial consortia demonstrated promising potential to support the ecological restoration of *S. toromiro* in Rapa Nui.

CAPÍTULO 1

INTRODUCCIÓN GENERAL

Sophora toromiro (Phil.) Skottsb., conocida comúnmente como toromiro, es una especie leguminosa endémica de la isla de Rapa Nui, Chile, que posee un alto valor cultural, espiritual y simbólico para el pueblo rapanui (Püschel, 2014). Su fruto era comestible y su madera fue utilizada en la elaboración de objetos ceremoniales y de uso cotidiano, tales como los moáis Kava-Kava y las tablillas Rongo-Rongo, en las cuales escribían (Pezoa et al., 2021). Con la llegada de colonos y el consiguiente aumento exponencial de la población insular (Wasilewski, 2012), sumado a la sobreexplotación del toromiro y la introducción de especies exóticas, provocaron la extinción del último ejemplar silvestre en 1960 (Espejo y Rodríguez, 2013). Actualmente, la especie se considera extinta en su hábitat natural, aunque persisten ejemplares en jardines botánicos europeos y en centros de conservación del territorio continental chileno (Maunder et al., 2000).

Diversas investigaciones han evidenciado que la deforestación histórica, junto con la pérdida progresiva de biodiversidad, ha provocado una transformación significativa en el paisaje de Rapa Nui (Mieth y Bork, 2005; Zizka y Zizka, 2022). Aunque se han desarrollado múltiples iniciativas orientadas a la reintroducción de *Sophora toromiro*, dichos esfuerzos no han logrado resultados exitosos hasta la fecha (Conservación del toromiro, 2025). Actualmente, se estima que aproximadamente el 60 % del territorio insular presenta algún grado de erosión del suelo (Flores et al., 2013), condición que, junto con la aparición de nuevas plagas, la escasez de bacterias fijadoras de nitrógeno, la limitada disponibilidad de macronutrientes esenciales como fósforo y potasio (Donoso y Schiappacasse, 2010; Ladefoged, 2010; CONAF, 2011; Benni, 2017), y la intensificación de eventos de sequía (Field et al., 2014), constituye un desafío para su restauración ecológica.

En las últimas décadas, el cambio climático ha sido ampliamente reconocido como una de las principales amenazas para la biodiversidad global (Pawson et al., 2013). Dentro de sus múltiples efectos, se ha documentado un aumento sostenido en la frecuencia e intensidad de eventos de sequía en distintas regiones del mundo (Garreaud et al., 2020; Mikiciuk et al., 2024). Rapa Nui presenta condiciones particularmente vulnerables frente al déficit hídrico, debido a su carácter insular, la ausencia de cuerpos de agua superficiales permanentes y su limitada capacidad de almacenamiento de agua dulce (DGA, 2010). La precipitación media anual es de 1.150 mm, con una temperatura promedio de 20,6 °C (Gatica y Toro, 2020). Sin embargo, el año 2023 se caracterizó por una drástica disminución de las precipitaciones, registrando solo 714,9 mm, lo que representa un 41,8 % por debajo del promedio histórico y lo posiciona como el sexto año más seco desde 1961 (Dirección Meteorológica de Chile, 2024). Las proyecciones climáticas indican una reducción adicional del 10 % en las precipitaciones hacia 2034 y del 25 % para el año 2064 (Gaymer et al., 2024), lo que agrava aún más el escenario para la restauración de especies nativas.

Diversas especies del género *Sophora* han mostrado alta sensibilidad a la escasez hídrica. En *Sophora davidii*, se ha documentado una reducción del 50 % en el área foliar y del 25 % en la altura de la planta bajo condiciones de humedad de suelo cercanas al 80 % de la capacidad de campo (Li et al., 2009). Por su parte, *Sophora moorcroftiana*, expuesta a 14 días de estrés hídrico continuo, presentó una disminución del 33 % en la tasa de fotosíntesis neta, junto con un incremento del 44 % en la concentración de malondialdehído (MDA) en las hojas, lo que evidencia el impacto bioquímico de este tipo de estrés (Guo et al., 2015). A pesar de ello, no se han realizado investigaciones que evalúen específicamente los efectos del déficit hídrico en *S. toromiro* ni en otras especies chilenas del género.

En la restauración de leguminosas, las bacterias fijadoras de nitrógeno juegan un rol central al establecer simbiosis específicas con sus plantas hospedadoras (Verma et al., 2020). Estos microorganismos, conocidos como rizobios, inducen la formación de nódulos en las raíces donde llevan a cabo la fijación biológica de nitrógeno atmosférico, aportando además vitaminas, aminoácidos, sideróforos y fitohormonas como las auxinas (Prasad et al., 2020). En el caso particular de *S. toromiro*, se ha demostrado que no es capaz de nodular con rizobios agrícolas convencionales, lo que subraya su especificidad simbiótica (Jordan et al., 2001). Estudios recientes han evidenciado que cepas del género *Mesorhizobium*, aisladas desde otras especies del mismo género como *S. macrocarpa* y *S. microphylla*, pueden establecer simbiosis efectivas con *S. toromiro*, mejorando significativamente la fijación de nitrógeno, el crecimiento de biomasa aérea y radicular, y la sobrevivencia en condiciones de vivero (Gerding y Espejo, 2019; Olave, 2020).

A pesar de estos avances, el papel de otros microorganismos benéficos, como las rizobacterias promotoras del crecimiento vegetal (PGPR), sigue siendo escasamente explorado en *Sophora*. Estas bacterias pueden beneficiar a las plantas a través de mecanismos directos, como la producción o modulación de fitohormonas (Adedeji et al., 2020), y la solubilización de nutrientes como fósforo y potasio, fundamentales para el desarrollo estructural y fisiológico vegetal (Etesami y Maheshwari, 2018).

La inoculación de consorcios bacterianos en plántulas puede generar efectos sinérgicos, como el aumento en la nodulación (Woo y Pepe, 2018), además de mejorar el estado fitosanitario de las plantas, permitiéndoles enfrentar situaciones de estrés (Zhu et al., 2019; Wang et al., 2022). Estas bacterias pueden aumentar la tolerancia a la sequía al promover el desarrollo del sistema radicular o reducir el estrés fisiológico mediante la producción de compuestos como ácido indol-3-acético o ácido indolacético (IAA) y la enzima ACC desaminasa (Ljung, 2013; Chamkhi et al., 2022).

Las interacciones microbianas en la rizósfera juegan un papel esencial en la mejora de la tolerancia vegetal al estrés abiótico, especialmente en contextos de restauración ecológica en ambientes degradados (Valliere et al., 2019). Si bien es sabido que los periodos prolongados de sequía reducen considerablemente la tasa de éxito en el establecimiento de plántulas, diversos estudios han demostrado que el uso de consorcios microbianos puede facilitar dicho proceso y mejorar las probabilidades de restauración (Valliere et al., 2020).

HIPOTESIS

La inoculación con consorcios bacterianos induce mayor crecimiento, nodulación, sobrevivencia, y tolerancia a estrés hídrico en plantas de *Sophora toromiro*.

OBJETIVO GENERAL

Evaluar el efecto de la inoculación de consorcios bacterianos conformados por rizobacterias sobre el crecimiento, nodulación, y tolerancia a estrés hídrico de *Sophora toromiro*.

OBJETIVOS ESPECÍFICOS

- Evaluar la habilidad de las rizobacterias asociadas a *Sophora* spp para solubilizar fósforo y potasio.
- Evaluar la compatibilidad entre *Mesorhizobium* sp. y rizobacterias solubilizadoras de fósforo y potasio para la conformación de consorcios microbianos para *Sophora toromiro*.
- Determinar el efecto de la inoculación de *Sophora toromiro* con consorcios microbianos en el crecimiento radicular, nodulación, y producción de biomasa.

- Determinar la influencia de los consorcios microbianos en el crecimiento y la tolerancia a estrés hídrico en plantas de *Sophora toromiro*.

REFERENCIAS

Adedeji, A. A., Häggblom, M. M., & Babalola, O. O. (2020). Sustainable agriculture in Africa: Plant growth-promoting rhizobacteria (PGPR) to the rescue. Scientific African, 9, e00492. https://doi.org/10.1016/j.sciaf.2020.e00492

Benni Montecino, Melanie. (2017). Análisis físico-químico de suelos utilizados para la recuperación de *Sophora toromiro*, especie endémico de Isla de Pascua. Tesis Técnico Universitario en Control del medio ambiente. Universidad técnica Federico Santa María. 128 pp.

Chamkhi, I., Omari, N. E., Balahbib, A., Menyiy, N. E., Benali, T., & Ghoulam, C. (2022). Is — — the rhizosphere a source of applicable multi-beneficial microorganisms for plant enhancement? Saudi Journal Of Biological Sciences, 29(2), 1246-1259. https://doi.org/10.1016/j.sjbs.2021.09.032

CONAF. (2011). Plan Nacional de Conservación del Toromiro, *Sophora toromiro*.

Conservación del toromiro. (2025). Iniciativas de reintroducción. Portal del Plan Nacional de Conservación *del Sophora toromiro*. https://plantoromiro.org/conservacion/iniciativas-de-reintroduccion/

DGA. (2010). Caracterización Hidrogeológica de la Isla de Pascua. Santiago: Dirección General de Aguas. Ministerio de Obras Públicas.

Dirección Meteorológica de Chile. (2024). Reporte anual de la evolución del clima en Chile: Informe climático del año 2023. Oficina Cambio Climático, Sección Climatología, Dirección General de Aeronáutica Civil.

Donoso, B., & Schiappacasse, F. (2010). *Sophora toromiro*, planta extinta en la vida silvestre: estudios micorrízicos, rizóbicos y nematológicos [Tesis de pre-grado, Universidad de Talca (Chile). Escuela de Agronomía]. http://dspace.utalca.cl/handle/1950/10360

Espejo, J., & Rodríguez, R. (2013). Antecedentes históricos en la descripción y del uso de *Sophora toromiro* (Phil.) Skottsb. (Fabaceae) en su hábitat natural: 1770-1948. Gayana. Botánica (Impresa), 70(2), 384-390. https://doi.org/10.4067/s0717-66432013000200015

Etesami, H., & Maheshwari, D. K. (2018). Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. Ecotoxicology And Environmental Safety, 156, 225-246. https://doi.org/10.1016/j.ecoenv.2018.03.013

F. Gaymer, C., Ramajo, L., Arthur, J., Aburto, J., González, G., Dewitte, B., Rivadeneira, M., & Bravo, L. (2024). Impactos, vulnerabilidad y capacidad de adaptación al cambio climático en Rapa Nui: hacia la identificación de fuentes de resiliencia a través de metodologías colaborativas (Version 1). figshare. https://doi.org/10.6084/m9.figshare.27287184.v1

Field, C.B. et al. (2014). Technical Summary. In: Climate Change 2014: Impacts,Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, et al. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 35-94.

Flores, J.P. Torres, M., Martínez, E., Muñoz, P. (2013). Determinación de la erosión actual y potencial de los suelos de Isla de Pascua. Informe final. Publicación n° 177 CIREN, Ministerio de Agricultura.

Garreaud, R. D., Boisier, J. P., Rondanelli, R., Montecinos, A., Sepúlveda, H. H., & Veloso-Aguila, D. (2020). The central Chile mega drought (2010–2018): a climate dynamics perspective. International Journal of Climatology, 40(1), 421-439.

Gatica, C. y Toro, I. (2020). Proyección de la Frecuencia de Eventos de Sequía para Rapa Nui, Escenarios de Cambio Climático RCP 4,5 y 8,5. XXIV Congreso Chileno de Ingeniería Hidráulica, Sociedad Chilena de Ingeniería Hidráulica. Santiago, Chile

Gerding M. y J. Espejo. (2019). Informe final "Selección de rizobios asociados a Sophora spp. para la inoculación y establecimiento de plantas de *Sophora toromiro* (Phil.) Skottsb. especie extinta en su hábitat natural". Convenio de Investigación Universidad de Concepción -Conaf Isla de Pascua (2017-2019). 22 pp.

Guo, Q., Zhang, W., & Li, H. (2014). Comparison of photosynthesis and antioxidative protection in *Sophora moorcroftiana* and Caragana maximovicziana under water stress. Journal of Arid Land, 6, 637-645. https://doi.org/10.1007/s40333-013-0212-9

Jordan, M., Larrain, M., Tapia, A., & Roveraro, C. (2001). In vitro regeneration of *Sophora toromiro* from seedling explants. Plant Cell, Tissue And Organ Culture, 66(2), 89-95. https://doi.org/10.1023/a:1010653418936

Ladefoged, T. N., Stevenson, C. M., Haoa, S., Mulrooney, M., Puleston, C. O., Vitousek, P. M., & Chadwick, O. A. (2010). Soil nutrient analysis of Rapa Nui gardening. Archaeology In Oceania/Archæology & Physical Anthropology In Oceania, 45(2), 80-85. https://doi.org/10.1002/j.1834-4453.2010.tb00082.x

Li, F. L., Bao, W. K., & Wu, N. (2009). Effects of water stress on growth, dry matter allocation and water-use efficiency of a leguminous species, *Sophora davidii*. Agroforestry systems, 77, 193-201. https://doi.org/10.1007/s10457-008-9199-1

Ljung, K. (2013). Auxin metabolism and homeostasis during plant development. Development (Cambridge), 140(5), 943-950. https://doi.org/10.1242/dev.086363

Maunder, M., Culham, A., Alden, B., Zizka, G., Orliac, C., Lobin, W., Bordeu, A., Ramirez, J. M., y Glissmann-Gough, S. 2000. Conservation of the Toromiro Tree: Case Study in the Management of a Plant Extinct in the Wild. Conserv. Biol., 14(5), 1341–1350.

Mieth, A., & Bork, H. (2005). History, origin and extent of soil erosion on Easter Island (Rapa Nui). Catena (Cremlingen), 63(2-3), 244-260. https://doi.org/10.1016/j.catena.2005.06.011

Mikiciuk, G., Miller, T., Kisiel, A., Cembrowska-Lech, D., Mikiciuk, M., Łobodzińska, A., & Bokszczanin, K. (2024). Harnessing Beneficial Microbes for Drought Tolerance: A

Review of Ecological and Agricultural Innovations. Agriculture, 14(12), 2228. https://doi.org/10.3390/agriculture14122228

Olave, I. (2020). Identificación y selección de rizobios para *Sophora toromiro* (Phil) Skottsb., especie extinta en Rapa nui. Tesis Ingeniero Agrónomo. Universidad de Concepción. 34 pp

Pawson, S. M., Brin, A., Brockerhoff, E. G., Lamb, D., Payn, T. W., Paquette, A., & Parrotta, J. A. (2013). Plantation forests, climate change and biodiversity. Biodiversity and Conservation, 22, 1203-1227. https://doi.org/10.1007/s10531-013-0458-8

Pezoa, I., Villacreses, J., Rubilar, M., Pizarro, C., Galleguillos, M. J., Ejsmentewicz, T., Fonseca, B., Espejo, J., Polanco, V., & Sánchez, C. (2021). Generation of Chloroplast Molecular Markers to Differentiate *Sophora toromiro* and Its Hybrids as a First Approach to Its Reintroduction in Rapa Nui (Easter Island). Plants, 10(2), 342. https://doi.org/10.3390/plants10020342

Prasad, R., Chhabra, S., Gill, S. S., Singh, P. K., & Tuteja, N. (2020). The microbial symbionts: Potential for crop improvement in changing environments. En Advancement in Crop Improvement Techniques (pp. 233-240). https://doi.org/10.1016/b978-0-12-818581-0.00014-0

Püschel, T. A., Espejo, J., Sanzana, M., & Benítez, H. A. (2014). Analysing the Floral Elements of the Lost Tree of Easter Island: A Morphometric Comparison between the Remaining Ex-Situ Lines of the Endemic Extinct Species *Sophora toromiro*. PloS One, 9(12), e115548. https://doi.org/10.1371/journal.pone.0115548

Valliere, J. M., Zhang, J., Sharifi, M. R., & Rundel, P. W. (2019). Can we condition native plants to increase drought tolerance and improve restoration success?. Ecological applications: a publication of the Ecological Society of America, 29(3), e01863. https://doi.org/10.1002/eap.1863.

Valliere, J. M., Wong, W. S., Nevill, P. G., Zhong, H., & Dixon, K. W. (2020). Preparing for the worst: Utilizing stress-tolerant soil microbial communities to aid ecological restoration in the Anthropocene. Ecological Solutions and Evidence, 1(2), e12027. https://doi.org/10.1002/2688-8319.12027

Verma, R., Annapragada, H., Katiyar, N., Shrutika, N., Das, K., & Senthilkumar, M. (2020). Chapter 4 - Rhizobium. En Beneficial Microbes in Agro-Ecology (pp. 37-54). https://doi.org/10.1016/b978-0-12-823414-3.00004-6

Wang, Y., Liu, H., Shen, Z., Yu, M., Wang, J., Jiang, X., & Shen, Q. (2022). Richness and antagonistic effects co-affect plant growth promotion by synthetic microbial consortia. Applied Soil Ecology, 170, 104300. https://doi.org/10.1016/j.apsoil.2021.104300

Wasilewski, M. 2012. Prehistory of Rapa Nui (Easter Island): a general outline.

Woo, S. L., & Pepe, O. (2018). Microbial Consortia: Promising Probiotics as Plant Biostimulants for Sustainable Agriculture. Frontiers In Plant Science, 9. https://doi.org/10.3389/fpls.2018.01801

Zhu, H., Liu, X., Zhang, F., & Yao, Q. (2019). Chapter 2 - Promoting Crop Growth With Symbiotic Microbes in Agro-Ecosystems in Climate Change Era. En New and Future Developments in Microbial Biotechnology and Bioengineering (pp. 31-41). https://doi.org/10.1016/b978-0-444-64191-5.00002-x

Zizka, G., Zizka, A. (2022). The Flora and Vegetation of Easter Island: Past and Present. In: Rull, V., Stevenson, C. (eds) The Prehistory of Rapa Nui (Easter Island). Developments in Paleoenvironmental Research, vol 22. Springer, Cham

CAPÍTULO 2

Growth-promoting and drought tolerance inducing bacterial consortia for *Sophora toromiro*, an extinct species from Rapa Nui Island

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Abstract

Sophora toromiro (Phil.) Skottsb. is a leguminous shrub endemic to Rapa Nui Island, Chile, currently extinct in its natural habitat. Its reintroduction is challenged by degraded soils, water scarcity, and the absence of compatible nitrogen-fixing bacteria. This study evaluated the potential of microbial consortia, composed by *Mesorhizobium* sp. and plant growth-promoting rhizobacteria (PGPR), to improve growth, nodulation and drought tolerance in *S. toromiro* propagated by stem cuttings. Bacterial strains were isolated from soils associated with *Sophora* species across Chile, including Rapa Nui and Magdalena Island, and were characterized and tested for compatibility. Greenhouse assays were conducted under controlled conditions, including a two-week drought period to simulate the island's seasonal water limitations. Inoculated treatments enhanced nodulation and showed favorable trends in root development compared to non-inoculated controls. Although no statistically significant differences were observed in other morphological parameters (main shoot height and growth, aerial and root dry weight and total biomass), these trends suggest a beneficial effect of co-inoculation, since some co-inoculated plants maintained the same relative root biomass under dry conditions. The use of vegetatively propagated plant material and the relatively short trial duration may have influenced our results, highlighting the need to consider these variables in future studies, especially in slow-growing species. Overall, the selection and application of compatible native bacterial consortia demonstrated promising potential to support the ecological restoration of *S. toromiro* in Rapa Nui.

Keywords: *Sophora toromiro*, ecological restoration, microbial consortia, PGPR, *Mesorhizobium*, drought tolerance, vegetative propagation.

1.Introduction

Sophora toromiro (Phil.) Skottsb., or 'toromiro', is a legume species endemic to Rapa Nui Island, Chile, that has an invaluable religious and cultural importance to islanders (Püschel, 2014). The wood of *Sophora toromiro* was used by the islanders to construct a variety of artifacts and objects, including the Kava-Kava moai and Rongo-Rongo tablets (Pezoa et al., 2021). Following the arrival of settlers on the island, the population grew exponentially (Wasilewski, 2012). This, combined with the over-exploitation of its timber and the introduction of exotic species, led to the extinction of the last remaining toromiro *in situ* in 1960 (Espejo and Rodríguez, 2013). Although the toromiro is currently considered extinct in its natural habitat, living specimens can be found in European botanical gardens and on the Chilean mainland (Maunder et al., 2000).

Deforestation and loss of biodiversity on Rapa Nui have negatively impacted on the natural habitat of the toromiro (Mieth and Bork, 2005; Zizka and Zizka, 2022). Despite several attempts to reintroduce the toromiro *in situ*, these have thus far been unsuccessful (Conservación del toromiro, 2025). Currently, approximately 60% of the island suffers from some degree of soil erosion (Flores et al., 2013). This, combined with new pests emerging, lack of nitrogen-fixing bacteria, low availability of certain macronutrients, such as phosphorus and potassium (Donoso and Schiappacasse, 2010; Ladefoged, 2010; CONAF, 2011; Benni, 2017), and intensifying droughts poses a significant challenge to restore the toromiro on the island (Field et al., 2014).

In recent decades, climate change has become an environmental concern and is expected to become one of the main drivers of biodiversity loss in the future (Pawson et al., 2013). Among the abiotic factors influenced by climate change, there has been a noticeable increase in the frequency and severity of drought events over time (Garreaud et al., 2020; Mikiciuk et al., 2024). Rapa Nui is an island with a limited area and an absence of permanent surface watercourses, thus rendering the supply of fresh water for various uses is a constant concern (DGA, 2010). The island's annual average rainfall is 1,150 mm, with a temperature of 20.6 °C (Gatica and Toro, 2020). However, in 2023, the annual rainfall was 41.8% below average, with a total of 714.9 mm recorded. This makes 2023 the sixth driest year since 1961 (Dirección Metereológica de Chile, 2024). Projections indicate a reduction in rainfall of close to 10% by 2034, while precipitation is projected to drop 25% by 2064 (Gaymer et al., 2024). Sophora spp plants have been reported to be highly sensitive to water scarcity. In *Sophora davidii*, leaf area and plant height were reduced by 50% and 25%, respectively, when soil water content was near 80% of field capacity (Li et al., 2009). In another study, *Sophora moorcroftiana* plants under water stress for 14 continuous days exhibited a reduction of 33% in net photosynthesis and an increase of 44% in MDA concentration in leaves, clearly showing the impact of water stress on plant biochemistry (Guo et al., 2014). Yet, there is no study addressing the role of water deprivation in *Sophora toromiro* and other Chilean *Sophora* spp.

A critical component of legume restoration is nitrogen-fixing bacteria, which are specific symbionts that form a mutualistic relationship with their host (Verma et al., 2020). Olave (2020) was able to select symbiotic rhizobia of the genus *Mesorhizobium* for inoculating them in toromiro, which showed positive effects on its growth under controlled conditions. Nevertheless, the selection of microorganisms that provide additional benefits, such as plant growth-promoting rhizobacteria, whose direct mechanisms include nutrient solubilization and phytohormone production (Babalola, 2010; Parewa et al., 2018), remains an unaddressed area of research. The formation and inoculation of bacterial consortia in seedlings with the aforementioned microorganisms not only has a synergistic effect among its individuals (Woo and Pepe, 2018), such as an increase in nodulation, but also improves the phytosanitary status of the plant, allowing it to cope with stress situations (Zhu et al., 2019; Wang et al., 2022). These microorganisms help the plant to resist drought stress by, for example, improving the root system or reducing stress levels through the production of compounds such as indoleacetic acid and the enzyme ACC deaminase (Ljung, 2013; Chamkhi et al., 2022).

Microbial interactions in the rhizosphere are key to improve plant tolerance to stress, particularly under field conditions in restoration sites (Valliere et al., 2019). Although periods of drought have been shown to reduce the success rate of plant establishment, the use of consortia composed by soil microorganisms has been shown to facilitate this process (Valliere et al., 2020). Therefore, the objectives of this study were to select bacteria with growth promoting attributes from the rhizosphere of *Sophora* spp. and to evaluate the effect of selected bacterial consortia on growth, drought tolerance and nodulation in *Sophora toromiro* plants propagated from cuttings.

2. Materials and Methods

2.1 Soil sampling

A total of 13 soil samples (500 g each) were collected for the isolation of bacteria from bulk soil and rhizosphere. Priority was given to areas where species of the *Sophora* genus could be found for sample collection. Ten samples were collected in continental and insular (Magdalena Island) Chile, and three were obtained specifically from Rapa Nui Island. The samples collected in continental Chile were associated with *Sophora macrocarpa* and *Sophora cassioides*. In the case of Magdalena Island, the samples were taken from pristine soils with high presence of vegetation. Soil samples from Rapa Nui were taken from areas where *S. toromiro* is being reintroduced (Miro Macoy 1 and 2, CONAF) and from Rano Kau volcano, where the last remaining specimen of this species was found (Skottsberg, 1920).

The QGIS program was used together with the "Luebert and Pliscoff 2017 vegetational floors" layer, obtained from geoportal.cl to determine the altitude and type of biome present in the different sampling areas (Table 2).

2.2 Isolation and selection of phosphorus and potassium-solubilizing bacteria

Soil suspensions were prepared by adding 10 g of each sample to 90 mL of a saline solution (0.9% w/v NaCl) and shaking for 15 min. Serial dilutions of 10^{-1} to 10^{-5} were prepared, and 0.1 mL were seeded into Petri dishes with Pikovskaya medium (Hariprasad and Niranjana, 2009) and modified Aleksandrov medium (Hu et al., 2006). The incubation period for the Pikovskaya plates was 48 hours, while the Aleksandrov plates were incubated for seven days at 25 degrees Celsius. Each pure culture was cryopreserved at a temperature of -80°C. The strains were reinoculated in each medium by depositing a 5 µL aliquot of bacterial broth with an optical density at 600 nm (OD_{600}) of 0.1, to measure the solubilization rate according to the method proposed by Premono et al. (1996), with the following formula:

Solubilization rate: Solubilization diameter (mm)/Colony diameter(mm)

2.3 Molecular fingerprinting

To identify isolates at the strain level, the BOX polymerase chain reaction (PCR) molecular technique was used (Versalovic et al., 1994), using the A1R partition (5'- CTA CGG CAA GGC GAC GCT GAC GAC G-3') as primer, according to the methodology of

Guiñazú et al. (2013). The cells used were centrifuged at 5,000 rpm for 5 min, washed 3 times, and adjusted to OD_{600}=1.5. They were then heat-shocked at 65°C for 5 min and then at -20°C for 5 min. The PCR reaction mixture contained 5.0 μL quenched cells, 0.3 μL Gotaq polymerase (Promega) (2 U μL^{-1}), 5.0 μL A1R primer (2 μM), 5.5 μL dNTPs (0. 2 mM), 2.5 μL Gotaq buffer (1x), 0.75 μL MgCl2 (1.5 mM), and 10.105 μL ultrapure water, totaling 25 μL (Marques et al. 2008). PCR conditions were: 95°C for 7 min, followed by 35 cycles at 94°C for 1 min, 52°C for 1 min, and 72°C for 8 min, and finally 72°C for 16 min (Guiñazú et al., 2013, with modifications). The products obtained from the PCR were analyzed by electrophoresis in a 2% (w/v) agarose gel with 2.5 μL mL-1 Gel Red (10,000x in DMSO, Biotium) applied over a 1x TAE buffer solution (40mM Tris-acetate, 1mM EDTA, pH 8.0), at a power of 75V for 3 h. After this time, the bands on the gel were observed in a UV transilluminator chamber.

2.4 16S rRNA gene sequencing

A PCR was performed to identify the isolates. The PCR reaction mixture consisted of 2 μL of bacterial culture, 2.5 units of Taq DNA polymerase (GoTaq®, Promega), 0.5 μM of forward and reverse primers, 1.5 mM MgCl2, 1X GoTaq® buffer, 0.2 mM dNTPs, and UltraPure grade water (Fisher Biotech), for a total volume of 50 μL for a single reaction. Cycling parameters were as follows: 95°C for five minutes, followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for one minute and 30 seconds. The final extension was conducted at 72°C for seven minutes.

PCR products were subjected to 1% (p/v) agarose gel electrophoresis and stained with GelRed nucleic acid gel stain (Biotium, Hayward, CA) for visualization. The purified amplicons were sequenced by Macrogen Inc. (Seoul, Korea) and used for phylogenetic analysis. The resulting chromatograms for the partial 16S rRNA sequences were analyzed and edited using Gene Tool Lite 1.0 software (2000) (Doubletwist, Inc., Oakland, CA, USA). Sequence alignments were used to identify the bacteria of interest using the BLAST search tool provided by the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/)

2.5 Indoleacetic acid production

To assess indoleacetic acid (IAA) production, selected strains were grown in nutrient broth; the OD_{600} was adjusted to 0.1 and the Salkowski colorimetric technique (Sarwar and Kremer, 1994) was used. A calibration curve was constructed by relating absorbance to IAA concentration ($R = 0.98$; $P < 0.001$).

2.6 ACC deaminase activity

To assess the ability of the strains to synthesize the enzyme ACC deaminase, 100 µL of cell suspension were transferred to nutrient broth at OD_{600}= 0.1 and incubated for 24 hours at 25 ± 2 °C, with shaking at 150 rpm. Subsequently, 1 mL was extracted and centrifuged for 10 minutes to remove the supernatant. The sample was then washed twice with 1 mL of Dworkin and Foster (DF) broth and left in 1 mL of the same broth with shaking. After 24 hours, the tubes were centrifuged (8000 g, 10 min) and 500 µL were removed to concentrate the bacterial suspension. From these tubes, 5 µL of each strain was extracted, and 9 strains were plated on 30 mL of DF agar, DF agar + ACC (without a nitrogen source), and DF agar + $(NH_4)^2SO_4$ 2 g (as a nitrogen source) (Penrose and Glick, 2003). Strains AG-29 (8R6WT) and AG-30 (YSS6WT) were used as positive controls, while strains AG-31 (8R6 acds-) and AG-32 (YSS6 acds-), which lack the acdS gene for ACC deaminase production, were used as negative controls (Rashid et al., 2012). The growth of colonies on DF basic medium was compared to the growth on DF medium with ACC, with respect to the growth of positive and negative controls.

2.7 Compatibility test

Compatibility tests were carried out with the selected bacterial strains and *Mesorhizobium* sp. strain AG-105 (Olave, 2020). Tubes containing 5 mL of culture broth -each inoculated with the selected bacterial strain- were prepared and shaken at 150 rpm for 24 hours at 25°C. After the incubation period, a 1 mL sample of the colonized broth was collected, centrifuged at 5,000 rpm for 5 minutes, and a cell wash was performed to remove the supernatant culture broth. A 1:10 dilution was then made by adding 100 µL of the cell suspension to 900 µL of saline solution (9% w/v). The OD_{600} was then measured and adjusted to 0.1. A bacterial lawn was established by plating 0.1 mL of each strain on a nutrient agar plate, with 5 µL of the adjusted strains plated in triplicate. Colony growth and inhibition halo formation were assessed at 24, 48, and 72 hours.

2.8 Effect of bacterial consortia inoculation

Plant material and growth conditions. *Sophora toromiro* plants (1-month-old cuttings) were provided by a nursery of CONAF (Corporación Nacional Forestal) - Chile. In general, *Sophora toromiro* plants are propagated by seeds to improve growth rates of roots, shoots and leaves. However, in the present study, plants were asexually propagated by cuttings, following the propagation protocols for *Sophora japonica* cuttings (Shams et al., 2015), to reduce the phenotypic variability of the resulting plants. The substrate was a combination of sand and vermiculite in a 1:1 ratio, which was sterilized three times in an autoclave. The plants were maintained in a phytotron at a temperature of 25 ± 3 °C and a photosynthetic photon flux density (PPFD) of 400-500 µmol m^{-2} s^{-1} using high-pressure sodium vapor lamps (400 W Gro-Lux®, Osram Sylvania Ltd., Danvers, MA, USA), with a 12 h photoperiod. Plants were irrigated three times per week with 20 mL of distilled water in order to maintain the substrate moisture between 70%

and 80% of container capacity. Additionally, every two weeks, plants were supplied with a modified legume nutrient solution containing immobilized phosphorus.

Experimental design. A randomized block design with 4 replicates and split-plot arrangement was established under the environmentally controlled conditions of the phytotron. Two irrigation treatments were applied as main plots. In the control treatment (WET), irrigation practices were adjusted to maintain substrate water content between 70% and 80% of container capacity throughout the experimental season. In the regulated deficit irrigation treatment (Dry), water was completely cut off for 14 consecutive days with the aim of simulating the rainfall regime of November, the driest month of the year (mean rainfall of 28.8 mm and rain event frequency of 15.8 days).

Five inoculation treatments were applied and used as subplots (Table 1). The inoculum preparation consisted in growing the selected strains and rhizobia in 4 mL tubes with nutrient broth under agitation (150 rpm) for 24 h at 25 °C, and the OD_{600} was adjusted to 0.1 in sucrose (1% v/v). The collar of each seedling was inoculated with 1 mL of standardized bacterial suspension (OD_{600} =0.1) as soon as plants were received from the CONAF nursery.

Evaluations

Three months after inoculation, the following traits were evaluated:

Nodulation. The number and position of nodules in the root system were counted, whether they were in the main root or in secondary roots. A nodule score was calculated from the position and size of the nodules (Yates et al., 2016)

Aerial and root dry weight. After nodulation was assessed, the aerial and root parts were separated and placed in paper bags and dried at 60°C for 48 hours to determine the dry weight of each experimental unit.

Leaf and root area. The Easy Leaf Area app was used to calculate the leaf area, and the ImageJ software (Abràmoff et al., 2004) used to calculate the root area.

Total biomass. Total biomass was calculated as follows:

Total biomass: Root Dry Weight (g) + Shoot Dry Weight

2.9 Statistical Analyses

Cluster analysis was performed using phosphorus and potassium solubilization indexes, followed by a comparison of clusters using Kruskal-Wallis nonparametric analysis of variance and Euclidean distance.

For strain determination by genetic fingerprinting, the amplified banding patterns were analyzed, and a binary matrix was constructed and analyzed in AFPL-SURV version 1.0 software (Vekemans, 2002). The genetic distance matrix was analyzed by UPGMA using the Neighbor application of the PHYLIP software package (Mansour, 2009). The cladogram was visualized in Mega 7 (Tamura et al., 2011), distinguishing individual strains to be used in subsequent assays and discarding copies.

The data obtained from the experiment were subjected to an analysis of variance (ANOVA) and a Fisher's test for comparison of means (LSD) with $p<0.05$. The variables that did not meet the assumptions of ANOVA were subjected to transformation and, when they did not meet the assumptions of the transformation, a non-parametric Kruskal-Wallis analysis of variance was performed. The statistical software InfoStat (Di Rienzo et al., 2016) was used for all the tests.

3. RESULTS

3.1 Isolation and selection of phosphorus and potassium-solubilizing bacteria

From the 13 soil samples collected (Table 1), 72 bacterial isolates were obtained, varying in colony morphology and in their ability to solubilize phosphorus and potassium. Of the 72 isolates, 54 were obtained from continental Chile (Puente Renegado: 3; La Campana: 7; Rucue: 16; Los Ruiles:8), 20 from Magdalena Island, and 18 from Rapa Nui (Rano Kau: 5: Miro Macoy:13).

Of the 72 isolates, 89% were able to solubilize phosphorus with an index ranging from 1.11 to 2.03. On the other hand, 60% were able to solubilize potassium with an index ranging from 1.07 to 2.14, and 49% of the isolates showed the ability to solubilize both phosphorus and potassium (Table 1, Supplementary Material). Bacteria with the ability to solubilize phosphorus and/or potassium were found at all sites, except in site 9 (S9) on Magdalena Island, where no potassium-solubilizing bacteria were present.

When analyzing the clusters of isolates based on their ability to solubilize phosphorus and potassium, four significantly different clusters were obtained according to the Euclidean distance. Fig. 1 shows the comparison between the four bacterial clusters based on their ability to solubilize phosphorus, where the cluster with the highest index was 4. This cluster was selected for testing and included an isolate from continental Chile (AG-329), specifically from a sample associated with *S. macrocarpa*, and the isolate AG-334 from Rapa Nui.

Cluster analysis of the isolates based on their potassium solubilization ability showed that clusters 3 and 4 had the highest solubilization indices, significantly higher than those of clusters 1 and 2 ($p \leq 0.05$; Fig. 2), leading to their selection for further testing. These clusters included 4 isolates from continental Chile (AG-327, AG-328, AG-

330 and AG-331), which were associated with *S. cassioides* and bulk soil and three isolates from Rapa Nui (AG-332, AG-334 and AG-335)

3.2 Molecular fingerprint

The bacterial isolates selected from the cluster analysis (Fig 1 and Fig 2) were all genetically distinct. This, according to cladogram analysis of genetic relationships based on RAPD-PCR products obtained with the A1R partition.

3.3 Identification through 16S rRNA gene sequencing

The strains were identified at the genus level through 16S rRNA gene sequencing. There were two strains identified within the *Pseudomonas* genus (AG-328 and AG-329); the strains AG-327, AG-331, AG-332, and AG-334 were identified as *Bacillus* spp. One strain was identified as *Rouxiella* spp. (AG-330) meanwhile, AG-333 as *Enterobacter* spp, and AG-335 as *Acinetobacter* spp.

3.4 ACC deaminase and IAA Production

Of the nine selected strains, five showed ACC deaminase activity, corresponding to the following strains: AG-328, AG-329, AG-330, AG-331, and AG-334. At the same time, AG-330 and AG-334 produced indole-3-acetic acid (IAA) at concentrations of 7.39 µg mL^{-1} and 3.68 µg mL^{-1}, respectively (Table 3).

3.5 Strain compatibility

The results of the compatibility test between *Mesorhizobium* sp. (AG-105) and the selected PGPR strains indicate that the strains compatible with the rhizobia were AG-329, AG-330, AG-333, and AG-335. In contrast, strains AG-327, AG-328, AG-331, AG-332, and AG-334 demonstrated antagonistic activity in the test.

3.6 Effect of bacterial consortia inoculation

Since there was no significant interaction between inoculation and irrigation treatments, irrigation data were pooled. Despite the lack of significant differences among inoculation treatments, inoculated plants tended to exhibit higher main shoot height and root and aerial dry biomass, in which the R + C2 treatment often show the highest values for vegetative growth parameters (Table 4).

Under well-watered conditions, statistically significant differences were observed among treatments in terms of nodule scoring ($p < 0.05$). All treatments enhanced nodulation compared to the control, except for R + C2, which showed no significant difference with the control. In contrast, no statistically significant differences were detected among treatments under drought conditions (Fig. 3).

Irrigation showed no effect on all vegetative growth parameters (Table 5). Yet, the main shoot growth of wet plants tended to be higher than dry plants (24%).

The relative root dry weight was significantly reduced by about 33% in dry plants that were inoculated with R and R + C1, while there were no significant differences between irrigation treatments for the remaining inoculation treatments (Fig. 4).

4. Discussion

The selection of bacterial inoculants to enhance plant growth, nutrition, and stress tolerance, is crucial for the re-establishment of *Sophora toromiro* in Rapa nui, given the droughts that have adversely affected the island over the past decade and the high soil erosion (Flores et al., 2013; Field et al., 2014¸ Benni, 2017). This study isolated a considerable number of bacterial strains with the ability to solubilize phosphorus and/or potassium. In legumes, such as *Sophora toromiro*, nitrogen is mainly provided through the symbiotic interaction with root nodule bacteria, or rhizobia (Velázquez et al., 2019).

However, phosphorus and potassium availability to plants largely depend on rhizosphere bacteria through a variety of mechanisms, including secretion of organic acids and enzymes, acidification of soils, pH reduction, and mineral chelation (Das and Pradhan, 2016; Meena et al., 2017; Nagargade et al., 2018; Billah et al., 2019). The selected strains for the consortia reached solubilization indexes that exceeded 1.5 for phosphorus and/potassium. The highest phosphorus solubilization index was exhibited by the *Pseudomonas* sp. strain AG-329, while the highest potassium solubilization index was exhibited by the *Acinetobacter* sp. strain AG-335. These bacterial genera are also known to act as biological control agents, plant growth promoters, and stress-tolerance inducers (Kumar et al., 2017; Kumari et al., 2018; Mujumdar et al., 2023).

Beyond nutrient solubilization, a functional consortium must also maintain internal microbial harmony to be effective in plant. A significant proportion of rhizosphere and soil bacteria can produce antibiotics and/or lytic enzymes; therefore, it is essential to evaluate the ability of bacterial strains to coexist in a consortium to prevent antagonistic effects (Olanrewaju et al., 2017; Velázquez et al., 2019). This observation is consistent with the results obtained in the *in vitro* compatibility tests, where only some PGPR were able to coexist in the culture medium without reducing *Mesorhizobium* sp. growth. This compatibility was also confirmed in the plant experiments, since effective nodulation was recorded for every co-inoculated treatment under wet conditions.

The use of indigenous soil bacteria as a strategy for ecological restoration has been shown to be more advantageous than the use of non-native inoculants, which may not be adapted to local conditions (Maltz and Treseder, 2015) and probably show less competitive abilities when introduced in natural microbiomes (Kong and Glick, 2017; Toju et al., 2018; Jiménez et al., 2020). In this study, native strains from Rapa nui (AG-333 and AG-335) were included in consortium 1 and 2 respectively, as they are likely to show greater adaptation to the target edaphoclimatic conditions. The strain AG-333 was identified as *Enterobacter hormaechi*, species that has been the focus of recent studies, with positive results in plant growth and stress tolerance (Roslan et al., 2020; Ranawat et al., 2021), and also in pathogen control (Wang et al., 2024).

Unlike single-strain inoculants, consortia are designed to carry out multiple complementary functions, enabling a more integrated and resilient response to various abiotic stressors (Jabborova et al., 2021; Abd El et al., 2022). For instance, each consortia used to inoculate *S. toromiro* included both strains that produced ACC deaminase (AG-329 and AG-330). The enzyme ACC deaminase, degrades the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), converting it into α-ketobutyrate and ammonia (Cedeño-García et al., 2018), decreasing ethylene concentration in soils and plants (Chieb and Gachomo.,2023). High concentrations of ethylene can inhibit plant growth, reduce leaf photochemical efficiency, and accelerate plant senescence (Van de Poel et al., 2014; Nazir et al., 2024). In legumes, ethylene is secreted by the plant as a defense response to rhizobial infection, disrupting early signaling and inhibiting nodule formation (Ligero et al., 1986; Mathesius, 2020; Gómez-Fernández et al., 2025). Although some rhizobia produce ACC deaminase to counteract this effect, it is often insufficient under stress conditions, leading to reduced nodulation and early nodule senescence (Ma et al., 2002; Ferguson and Mathesius, 2014). Since the development of root nodules is essential for biological nitrogen fixation and plant growth promotion (Kaschuk et al., 2009; Yeremko et al., 2025), non-rhizobial bacteria with ACC-deaminase activity can be of paramount importance to delay nodule senescence under stress conditions (Nascimento et al., 2016; Hamid et al., 2021; Zhao et al., 2023). In our study, the inclusion of AG-329 and AG-330 in the consortia showed no improvement on nodulation or vegetative growth parameters under wet and dry soil conditions compared to plants inoculated with the *Mesorhizobium* strain alone. Yet, plants inoculated with consortia 1 and 2 tended to show increased dry root mass, despite the lack of significant differences

The *Mesorhizobium* sp. AG-105 plays a well-established role in forming symbiotic associations with legumes through the development of specialized root nodules, which is essential for biological nitrogen fixation (Kaschuk et al., 2009; Yeremko et al., 2025). Consequently, Mesorhizobium sp. should be a central component of consortia for the inoculation of *Sophora toromiro*. Surprisingly, after 14 days under dry conditions, plants inoculated with *Mesorhizobium* alone and in combination with consortia 1 showed significant reductions in relative root dry weight (-33%) (Figure 4). The symbiotic nodule formation and activity are highly dependent on plant carbohydrate supply, which can vary between 4% and 25% of total photosynthate production (Lepetit and Brouquisse, 2023). Under water stress conditions, net photosynthesis of *Sophora* plants can be reduced by 33% after 14 continuous days (Guo et al., 2014), which can strongly limit carbohydrate supply. Often, legumes reduce nodulation or sacrifice nodules to prioritize root development when stress conditions reduce carbohydrate production (Liao et al., 2023). However, in certain cases, such as in water-stressed toromiro plants inoculated with *Mesorhizobium* alone and in combination with consortia 1, the maintenance of functional nodules may be prioritized over root development (Marino et al., 2007; Dhanushkodi et al., 2018; Pholo et al., 2022). To reduce the physiological costs associated with nodulation, co-inoculation with PGPR has proven to be an effective strategy (Vishwakarma et al., 2020) that can enhance physiological and growth responses in legumes, improving nodulation and nutrient uptake (Chauhan and Bagyaraj, 2015; Zaidi, 2017; Kumar et al., 2022; Shome et al., 2022). In this work, although nodulation score was similar among all inoculated treatments, plants inoculated with consortia 2 and 3 showed no reduction in relative root biomass under dry conditions. Unlike consortia 1, consortia 2 and consortia 3 include *Rouxiella* sp. AG-330, a strain that has shown ACC-deaminase activity and indoleacetic acid (IAA) production. The bacterial synthesis of IAA can promote root elongation and the formation of lateral roots (Etesami and Maheshawari, 2018), which may explain the maintenance of relative root biomass in plants inoculated with consortia 2 and 3.

Two important aspects of this study are the propagation strategy of the plant material and the length of the experiment. *Sophora toromiro* has been sexually and asexually propagated to preserve *ex situ* populations in botanical gardens and nurseries (Jordan et al., 2010; Espejo et al., 2013; Vásquez et al., 2020). However, there are no microbiological or physiological studies on toromiro plants propagated by stem cuttings, as is the case in our research. This method of vegetative propagation allows the production of genetically identical clones (Yadav and Singh, 2018), which is particularly relevant to preserve the genetic purity of *Sophora toromiro* for restoration purposes. The success of this propagation technique largely depends on the non-structural carbohydrate reserves present in the tissue at the time of cutting. These reserves are influenced by several factors, including the physiological age of the donor plant, its nutritional status, the time of year, and the specific segment selected (Tsafouros et al., 2019; De lima et al., 2020; Dwumah et al., 2024). In this study, thin cuttings were used, which may have influenced the observed outcomes, since this type of material generally contains fewer carbohydrate and nutrient reserves than more robust cuttings. This limitation can affect root initiation and aerial growth because energy must be mobilized to sustain both processes simultaneously, especially during the early stages, before the plant establishes sufficient photosynthetic capacity (Kaczmarek et al., 2014; Lorh et al., 2017; Souza et al., 2019;). Witt et al., (2023) reported a decrease in nodulation in legumes propagated from cuttings. However, other studies have shown beneficial effects of PGPR inoculation on root development, increasing their chances of survival (Erturk et al., 2010; Zulfitri, 2012; Nwigwe, 2018; Dalda-Skerci et al., 2024).

Sophora toromiro is a slow-growing leguminous shrub (Iturriaga et al., 1994). Slow-growing legume species often show delayed establishment of symbiotic relationships with nitrogen fixing rhizobia (Simonsen et al., 2017), which may explain the lack of statistically significant differences observed in this short-term study. Similar outcomes have been reported on other leguminous shrub species such as *Acacia decurrens* (Sánchez and Bonilla, 2014), *Acacia farnesiana*, and *Prosopis glandulosa* (Hernández-Martínez et al., 2006), where trends rather than clear differences emerged in experiments lasting 20–126 days. In contrast, longer studies on *Sophora davidii* (Wu et al., 2008; Gong et al., 2013) and *Prosopis laevigata* (Crespo-Flores et al., 2021), found statistically significant effects of inoculation, highlighting the importance of trial duration. Future studies should prioritize longer evaluation periods and field-based trials to validate these findings and optimize the application of microbial consortia tailored to local edaphoclimatic conditions.

5. Conclusions

This study demonstrated that native bacteria with growth-promoting properties can be found in soils associated with *Sophora* spp. and soils from Rapa Nui Island, Chile. The inoculation of *Sophora toromiro* with native bacterial consortia, including plant growth promoting bacteria and rhizobia, showed potential to enhance nodulation and improve root development, particularly under drought conditions. The use of stem cuttings as propagation material introduces specific physiological challenges that may influence plant-microbe interactions, since limited carbohydrate reserves and delayed root establishment may have reduced the impact of inoculation in the short term.

Trial duration is a critical factor when evaluating plant-microbe interactions in slow-growing species. Future research should incorporate longer experimental periods to fully understand the benefits of microbial inoculation in *S. toromiro* and similar species.

Nevertheless, the selection and inoculation of native and functional microbial consortia represent a promising strategy to support the ecological reintroduction of *Sophora toromiro* into its native habitat.

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Author contribution

D.A. performed the experiments, analyzed the data and wrote the first version of the manuscript; M.G. and A.C.O. designed and managed the experiments, analyzed the data, and drafted and finalized the manuscript; L.P, C.M, P.O and J.E contributed to the design of the experiments and edited the final version of the manuscript.

All authors have read and agreed to the published version of the manuscript.

Statements and Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Figure 1. Comparison between bacterial clusters based on the phosphorus solubilization index. Vertical bars correspond to the standard error of the mean. Means sharing a letter are not significantly different, according to the Kruskal-Wallis test ($p < 0.05$).

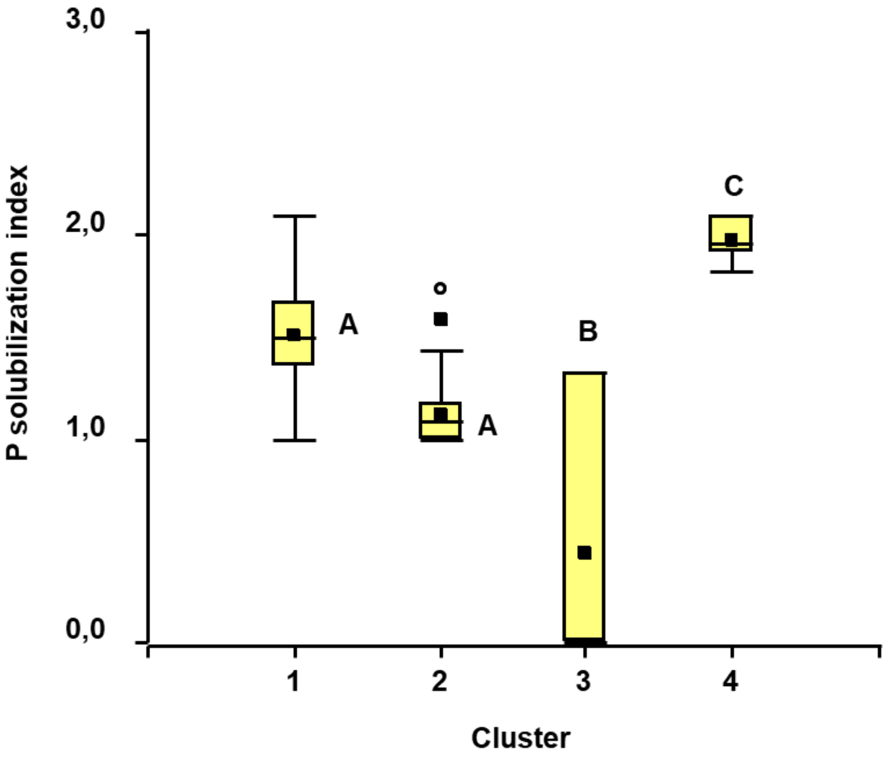

Figure 2. Comparison between bacterial clusters based on the potassium solubilization index. Vertical bars correspond to the standard error of the mean. Means sharing a letter are not significantly different, according to the Kruskal-Wallis test ($p < 0.05$).

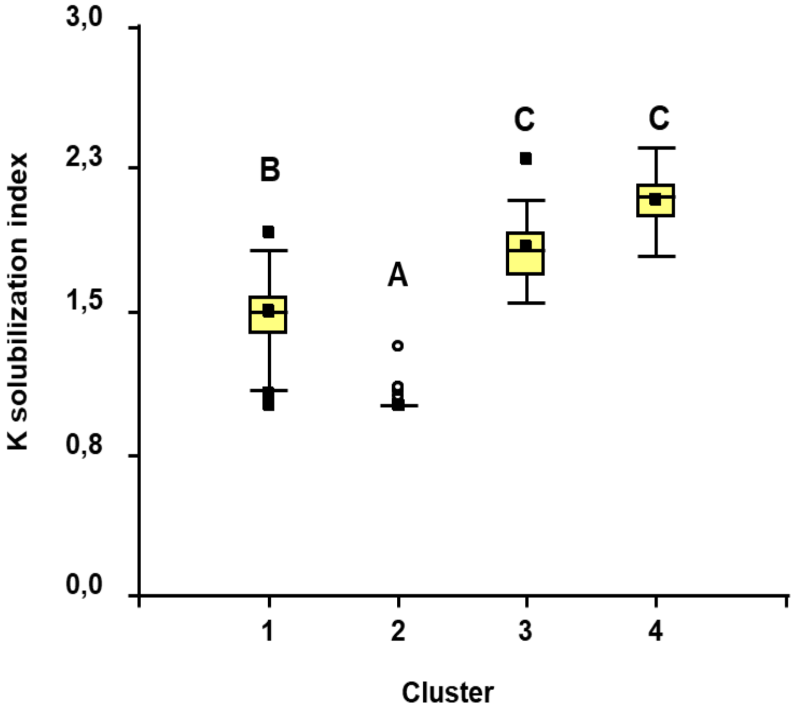

Figure 3. Nodule scoring in *Sophora toromiro* seedlings inoculated with different bacterial treatments under two watering conditions. Bars represent the mean ± standard error. Statistical analysis was conducted independently within each watering condition. Different letters indicate statistically significant differences among treatments within the same condition, according to one-way ANOVA followed by Fisher's LSD test (p ≤ 0.05)

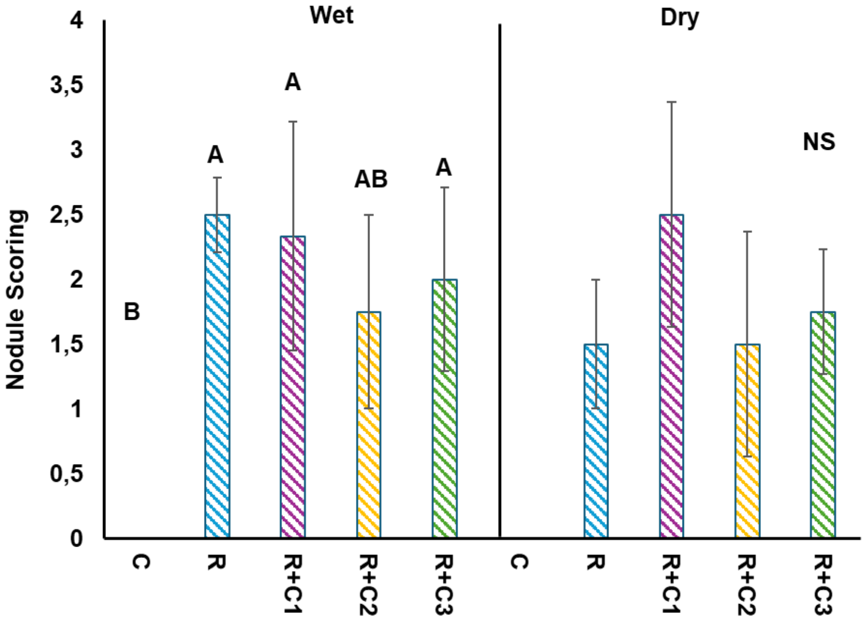

Figure 4. Comparison of relative root weight between irrigation conditions per treatment. Different letters indicate significant differences between irrigation conditions within the same treatment, according to one-way ANOVA followed by Fisher's LSD test (p ≤ 0.05)

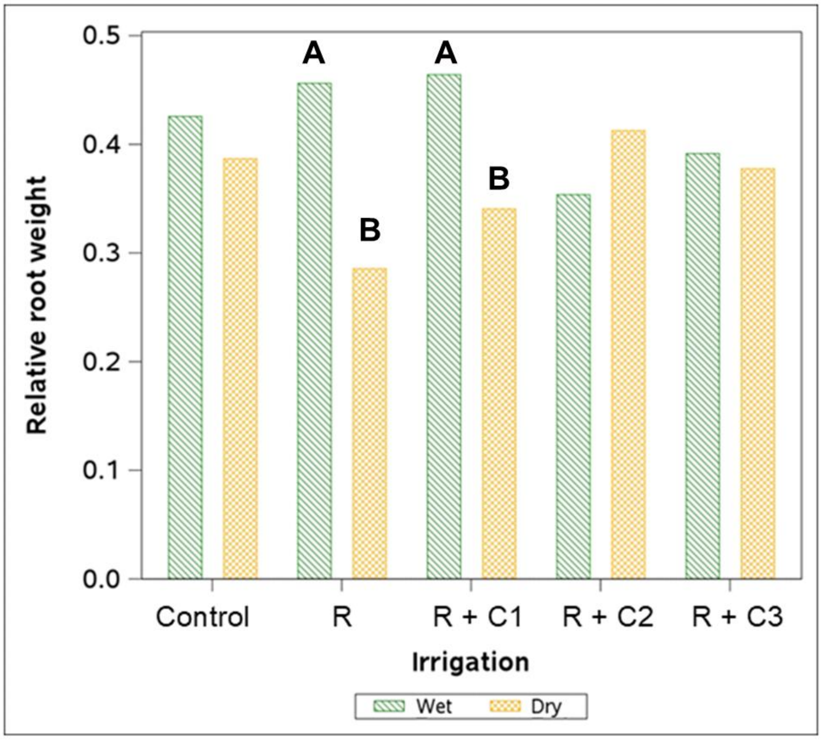

Table 1. Conformation of treatments used in the experiment.

Treatment	Abbreviation	Conformation
Control	C	Plants without inoculation
Rhizobia	R	*Mesorhizobium* sp, AG-105
Consortium 1	R + C1	*Mesorhizobium* sp (AG-105), PGPR (AG-329 and AG-333).
Consortium 2	R + C2	*Mesorhizobium* sp. (AG-105), PGPR (AG-330 and AG-335)
Consortium 3	R + C3	*Mesorhizobium* sp (AG-105), PGPR (AG-330)

Table 2. Geographic and ecological characteristics of soil sampling sites, including location, coordinates, altitude, biome type and number of isolates.

Sample	Location	Altitude	Biome	Coordinates	N° Isolates
S1	Puente Renegado (C)	790	Deciduous forest	38°51'25.62"S 71°38'20.1"W	3
S2	La Campana (C)	694	Deciduous forest	32°57'12.0"S 71°04´00.2"W	7
S3	Rucue (C)	460	Deciduous forest	37°20'36.2"S 71°47'53.0"W	2
S4	Rucue (C)	460	Deciduous forest	37°20'36.2"S 71°47'53.0"W	8
S5	Rucue (C)	460	Deciduous forest	37°20'36.2"S 71°47'53.0"W	6
S6	Los Ruiles (C)	365	Deciduous forest	35°49'32.1"S 72°22'28.5"W	5
S7	Los Ruiles (C)	365	Deciduous forest	35°49'32.1"S 72°22'28.5"W	3
S8	Isla Magdalena (I)	256	ephemeral grassland	52°55'6.9"S 70°34'40.1"W	9
S9	Isla Magdalena (I)	256	ephemeral grassland	52°55'6.9"S 70°34'40.1"W	6
S10	Isla Magdalena (I)	256	ephemeral grassland	52°55'6.9"S 70°34'40.1"W	5
S11	Miro Macoy 1 (R)	23	Steppes and grasslands	27°6'8.5"S 109°20'47.0"W	11
S12	Miro Macoy 2 (R)	23	Steppes and grasslands	27°6'8.5"S 109°20'47.0"W	2
S13	Rano Kau (R)	66	Shrub	27°07′15.1″S 109°17′31.6″W	5

(C): Continental Chile; (I): Insular Chile; (R): Rapa Nui.

Table 3. Characterization of selected bacteria in ACC-Desaminase activity and indoleacetic acid (IAA) production.

Strain	Location	Origin	PSI	KSI	ACC-Desaminase Activity	IAA production (µg mL-1)
AG-327	S4	*S.cassioides*	1.39	1.87	-	0
AG-328	S5	*S.cassioides*	1.54	1.81	+	0
AG-329	S6	*S.macrocarpa*	2.03	1.61	+	0
AG-330	S7	Soil	1.53	1.91	+	7.39
AG-331	S10	Soil	1.53	1.78	+	0
AG-333	S11	Soil	1	1.82	-	0
AG-332	S11	Soil	1.77	1.92	-	0
AG-335	S13	Soil	1.48	2.14	-	0
AG-334	S13	Soil	1.92	1	+	3.68

PSI: phosphorus solubilization index; KSI: potassium solubilization index.

Table 4. Vegetative growth parameters in *Sophora toromiro* plants inoculated with different bacterial treatments (C: Control, R: *Rhizobium*, R+C1, R+C2, R+C3). Different letters indicate significant differences between treatments, according to one-way ANOVA followed by Fisher's LSD test ($p \leq 0.05$). Values represent means ± standard error.

Treatments	Main Shoot Height (cm)	Main shoot growth (cm)	Aerial Dry weight (g)	Root Dry weight (g)	Total biomass (g)
C	9.30 ± 0.70	2.56 ± 0.53	0.20 ± 0.02	0.13 ± 0.01	0.34 ± 0.03
R	10.10 ± 0.73	2.45 ± 0.48	0.23 ± 0.03	0.15 ± 0.03	0.38 ± 0.06
R + C1	10.87 ± 0.67	3.81 ± 0.66	0.27 ± 0.04	0.18 ± 0.01	0.45 ± 0.05
R + C2	11.46 ± 0.67	3.46 ± 0.87	0.26 ± 0.02	0.21 ± 0.05	0.47 ± 0.06
R + C3	10.43 ± 0.41	3.06 ± 0.54	0.25 ± 0.03	0.16 ± 0.02	0.42 ± 0.04

Table 5. Vegetative growth parameters in Sophora toromiro under two irrigation conditions (Wet and Dry). Different letters indicate significant differences between irrigation conditions, according to one-way ANOVA followed by Fisher's LSD test ($p \leq 0.05$). Values represent means ± standard error.

Condition	Main Shoot Height (cm)	Main shoot growth (cm)	Aerial Dry weight (g)	Root Dry weight (g)	Total biomass (g)
Wet	10.55 ± 0.43	3.49 ± 0.36	0.24 ± 0.02	0.17 ± 0.01	0.41 ± 0.03
Dry	10.32 ± 0.42	2.65 ± 0.41	0.24 ± 0.01	0.16 ± 0.02	0.41 ± 0.03

References

Abd El-Malik Mohamed Abd El-Azeem, S. (2022). Effects of Co-Inoculation of Rhizobium and Plant Growth-Promoting Rhizobacteria on Common Bean (*Phaseolus vulgaris*) Yield, Nodulation, Nutrient Uptake, and Microbial Activity under Field Conditions. *Journal of Soil and Water Sciences*, *7*(1), 13-26. doi: 10.21608/jsws.2022.290563

Abràmoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics international*, *11*(7), 36-42

Babalola, O. O. (2010). Beneficial bacteria of agricultural importance. Biotechnology Letters (Print), 32(11), 1559-1570. https://doi.org/10.1007/s10529-010-0347-0

Benni Montecino, Melanie. (2017). Análisis físico-químico de suelos utilizados para la recuperación de *Sophora toromiro*, especie endémico de Isla de Pascua. Tesis Técnico Universitario en Control del medio ambiente. Universidad técnica Federico Santa María. 128 pp.

Billah, M., Khan, M., Bano, A., Hassan, T. U., Munir, A., & Gurmani, A. R. (2019). Phosphorus and phosphate solubilizing bacteria: Keys for sustainable agriculture. Geomicrobiology Journal, 36(10), 904–916. https://doi.org/10.1080/01490451.2019.1654043

Cedeño-García, George A., Gerding, Macarena, Moraga, Gary, Inostroza, Luis, Fischer, Susana, Sepúlveda-Caamaño, Maurine, & Oyarzúa, Pía. (2018). Plant growth promoting rhizobacteria with ACC deaminase activity isolated from Mediterranean dryland areas in Chile: Effects on early nodulation in alfalfa. Chilean journal of agricultural research, *78*(3), 360-369. https://dx.doi.org/10.4067/S0718-58392018000300360

Chamkhi, I., El Omari, N., Balahbib, A., El Menyiy, N., Benali, T., y Ghoulam, C. (2022). Is —— the rhizosphere a source of applicable multi-beneficial microorganisms for plant enhancement? Saudi J Biol Sci., 29(2), 1246-1259. https://doi.org/10.1016/j.sjbs.2021.09.032

Chauhan, H., & Bagyaraj, D. J. (2015). Inoculation with selected microbial consortia not only enhances growth and yield of French bean but also reduces fertilizer application under field condition. Scientia Horticulturae, *197*, 441-446. https://doi.org/https://doi.org/10.1016/j.scienta.2015.10.001

Chieb, M., & Gachomo, E. W. (2023). The role of plant growth promoting rhizobacteria in plant drought stress responses. BMC plant biology, *23*(1), 407. https://doi.org/10.1186/s12870-023-04403-8

CONAF. (2011). Plan Nacional de Conservación del Toromiro, Sophora toromiro.

Conservación del toromiro. (2025). Iniciativas de reintroducción. Portal del Plan Nacional de Conservación del *Sophora toromiro*. https://plantoromiro.org/conservacion/iniciativas-de-reintroduccion/

Crespo Flores, Gustavo, Ramírez Tobias, Hugo M., Vallejo Pérez, Moisés R., & Méndez Cortés, Heriberto. (2021). Co-inoculation with rhizobia and arbuscular mycorrhizal fungi in seedlings of *Prosopis laevigata*. Revista mexicana de ciencias agrícolas, *12*(7), 1249-1262.https://doi.org/10.29312/remexca.v12i7.2910

DALDA-SEKERCİ, A., KANATBEKOVA, K., & UNLU, E. (2024). Assessment of the Effect of Plant Growth Promoting Rhizobacteria (PGPR) Strains on Rooting in Rose Cuttings. *Assessment*, *4*(2). https://dx.doi.org/https://doi.org/10.54498/ETBD.2024.34

Das, I., Pradhan, M. (2016). Potassium-Solubilizing Microorganisms and Their Role in Enhancing Soil Fertility and Health. In: Meena, V., Maurya, B., Verma, J., Meena, R. (eds) Potassium Solubilizing Microorganisms for Sustainable Agriculture. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2776-2_20

De Lima, A. F., Lins, M. M., De Souza, C. G., dos Santos, M. V. F., Ferreira, R. L. C., Da Cunha, M. V., ... & De Souza, T. C. (2020). Development, rooting and nodulation of mororó (*Bauhinia cheilantha*) cuttings harvested in different seasons. Tropical Grasslands-Forrajes

DGA. (2010). Caracterización Hidrogeológica de la Isla de Pascua. Santiago: Dirección General de Aguas. Ministerio de Obras Públicas.

Dhanushkodi, R., Matthew, C., McManus, M. T., & Dijkwel, P. P. (2018). Drought-induced senescence of *Medicago truncatula* nodules involves serpin and ferritin to control proteolytic activity and iron levels. New Phytologist, 220(1), 196-208. https://doi.org/10.1111/nph.15298

Di Rienzo J.A., F. Casanoves, M.G. Balzarini, L. Gonzalez, M. Tablada and C.W. Robledo. (2016). InfoStat versión 2016. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina

Dirección Meteorológica de Chile. (2024). *Reporte anual de la evolución del clima en Chile: Informe climático del año 2023*. Oficina Cambio Climático, Sección Climatología, Dirección General de Aeronáutica Civil.

Donoso, B., & Schiappacasse, F. (2010). *Sophora toromiro*, planta extinta en la vida silvestre: estudios micorrízicos, rizóbicos y nematológicos [Tesis de pre-grado, Universidad de Talca (Chile). Escuela de Agronomía]. http://dspace.utalca.cl/handle/1950/10360

Dwumah, K. A., Boadi, S., & Kyereh, B. (2024). Clonal Propagation of Broussonetia Papyrifera by Stem and Root Cuttings. Journal of Science and Technology (Ghana), 1(2), 1-12. https://doi.org/10.4314/just.v1i2.4s

ERTURK, YASAR, ERCISLI, SEZAI, HAZNEDAR, AYHAN, & CAKMAKCI, RAMAZAN. (2010). Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. Biological Research, 43(1), 91-98. https://dx.doi.org/10.4067/S0716-97602010000100011

Equipo de desarrollo de QGIS (2025). Sistema de información geográfica de QGIS. Proyecto de la Fundación Geoespacial de código abierto. http://qgis.osgeo.org

Espejo, J., & Rodríguez, R. (2013). Antecedentes históricos en la descripción y del uso de Sophora toromiro (Phil.) Skottsb. (Fabaceae) en su hábitat natural: 1770-1948. Gayana. BotáNica (Impresa), 70(2), 384-390. https://doi.org/10.4067/s0717-66432013000200015

Espejo, Jaime, Baeza, Marcelo, Ruiz, Eduardo, Mora, Freddy, Gomez, Miguel, & Montenegro, Gloria. (2013). Propagation of *Sophora toromiro* through interspecific grafting to support species conservation: Masificación de *Sophora toromiro*, mediante injerto inter específico como apoyo a la conservación de la especie. *Ciencia e investigación agraria*, *40*(1), 213-221. https://dx.doi.org/10.4067/S0718-16202013000100019

Etesami, H., & Maheshwari, D. K. (2018). Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. Ecotoxicology and environmental safety, 156, 225–246. https://doi.org/10.1016/j.ecoenv.2018.03.013.

Ferguson, B. J., & Mathesius, U. (2014). Phytohormone regulation of legume-rhizobia interactions. *Journal of chemical ecology*, *40*(7), 770–790. https://doi.org/10.1007/s10886-014-0472-7

Field, C.B. et al. (2014). Technical Summary. In: Climate Change 2014: Impacts,Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, et al. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 35-94.

Flores, J.P. Torres, M., Martínez, E., Muñoz, P. (2013). Determinación de la erosión actual y potencial de los suelos de Isla de Pascua. Informe final. Publicación N° 177 CIREN, Ministerio de Agricultura.

Garreaud, R. D., Boisier, J. P., Rondanelli, R., Montecinos, A., Sepúlveda, H. H., & Veloso-Aguila, D. (2020). The central Chile mega drought (2010–2018): a climate dynamics perspective. International Journal of Climatology, 40(1), 421-439.

Gatica, C. y Toro, I. (2020). Proyección de la Frecuencia de Eventos de Sequía para Rapa Nui, Escenarios de Cambio Climático RCP 4,5 y 8,5. XXIV Congreso Chileno de Ingeniería Hidráulica, Sociedad Chilena de Ingeniería Hidráulica. Santiago, Chile

F. Gaymer, C., Ramajo, L., Arthur, J., Aburto, J., González, G., Dewitte, B., Rivadeneira, M., & Bravo, L. (2024). Impactos, vulnerabilidad y capacidad de adaptación al cambio climático en Rapa Nui: hacia la identificación de fuentes de resiliencia a través de metodologías colaborativas (Version 1). figshare. https://doi.org/10.6084/m9.figshare.27287184.v1

Gómez-Fernández, G. O., van Velzen, R., Mun, J. H., Cook, D. R., Kohlen, W., & Larrainzar, E. (2025). Ethylene biosynthesis in legumes: gene identification and expression during early symbiotic stages. Journal of Experimental Botany, eraf069. https://doi.org/10.1093/jxb/eraf069

Gong, M., Tang, M., Chen, H. *et al.* Effects of two *Glomus* species on the growth and physiological performance of *Sophora davidii* seedlings under water stress. New Forests **44**, 399–408 (2013). https://doi.org/10.1007/s11056-012-9349-1

Guiñazú, L. B., Andrés, J. A., Rovera, M., Balzarini, M., y Rosas, S. B. (2013). Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia* sp. and *Macrophomina* sp. in vitro. Eur. J. Soil Biol, 54, 69-77. https://doi.org/10.1016/j.ejsobi.2012.09.007

Guo, Q., Zhang, W., & Li, H. (2014). Comparison of photosynthesis and antioxidative protection in *Sophora moorcroftiana* and *Caragana maximovicziana* under water stress. Journal of Arid Land, *6*, 637-645. https://doi.org/10.1007/s40333-013-0212-9

Hamid, B., Zaman, M., Farooq, S., Fatima, S., Sayyed, R. Z., Baba, Z. A., ... & Suriani, N. L. (2021). Bacterial plant biostimulants: a sustainable way towards improving growth, productivity, and health of crops. Sustainability., 13(5), 2856. https://doi.org/10.3390/su13052856

Hariprasad, P., y Niranjana, S. R. (2009). Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant Soil., 316, 13-24. https://doi.org/10.1007/s11104-008-9754-6

Hernández-Martínez, M., Cetina-Alcalá, V. M., González-Chávez, M. C., & Cervantes-Martínez, C. T. (2006). Inoculación micorrízica y su efecto en el crecimiento de dos leguminosas arbóreas. *Terra Latinoamericana*, *24*(1), 65-73.

Hu, X., Chen, J., y Guo, J. (2006). Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World J. Microbiol. Biotechnol., 22(9), 983-990. https://doi.org/10.1007/s11274-006-9144-2

Iturriaga, L., Jordan, M., Roveraro, C., & Goreux, A. (1994). In vitro culture of Sophora toromiro (Papilionaceae), an endangered species. *Plant cell, tissue and organ culture*, *37*, 201-204.

Jabborova, D., Kannepalli, A., Davranov, K., Narimanov, A., Enakiev, Y., Syed, A., ... & Gafur, A. (2021). Co-inoculation of rhizobacteria promotes growth, yield, and nutrient contents in soybean and improves soil enzymes and nutrients under drought conditions. Scientific Reports, 11(1), 22081. https://doi.org/10.1038/s41598-021-01337-9

Jiménez JA, Novinscak A and Filion M. (2020). Inoculation With the Plant-Growth-Promoting Rhizobacterium *Pseudomonas fluorescens* LBUM677 Impacts the Rhizosphere Microbiome of Three Oilseed Crops. *Front.* Microbiol. 11:569366. doi: 10.3389/fmicb.2020.569366

Jordan, M., Larrain, M., Tapia, A., & Roveraro, C. (2001). In vitro regeneration of Sophora toromiro from seedling explants. *Plant cell, tissue and organ culture*, *66*, 89-95. https://doi.org/10.1023/A:1010653418936

Kaczmarek, D. J., Rousseau, R. J., Wright, J. A., & Wachelka, B. C. (2014). The influence of alternative plant propagation and stand establishment techniques on survival and growth of eastern cottonwood (*Populus deltoides* Bartr.) clones. *New Forests*, *45*, 487-506. https://doi.org/10.1007/s11056-014-9411-2.

Kaschuk, G., Kuyper, T. W., Leffelaar, P. A., Hungria, M., & Giller, K. E. (2009). Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses?. Soil Biology and Biochemistry, *41*(6), 1233-1244. https://doi.org/10.1016/j.soilbio.2009.03.005

Kong, Z., & Glick, B. R. (2017). The Role of Plant Growth-Promoting Bacteria in Metal Phytoremediation. Advances in microbial physiology, 71, 97–132. https://doi.org/10.1016/bs.ampbs.2017.04.001

Kumar, A. et al. (2017). Role of *Pseudomonas* sp. in Sustainable Agriculture and Disease Management. In: Meena, V., Mishra, P., Bisht, J., Pattanayak, A. (eds) Agriculturally Important Microbes for Sustainable Agriculture. Springer, Singapore. https://doi.org/10.1007/978-981-10-5343-6_7

Kumar, S., Sindhu, S. S., & Kumar, R. (2022). Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. Current Research in Microbial Sciences, 3, 100094. https://doi.org/10.1016/j.crmicr.2021.100094

Kumari, P., Meena, M., & Upadhyay, R. (2018). Characterization of plant growth promoting rhizobacteria (PGPR) isolated from the rhizosphere of *Vigna radiata* (mung bean). Biocatalysis And Agricultural Biotechnology, 16, 155-162. https://doi.org/10.1016/j.bcab.2018.07.029

Ladefoged, T. N., Stevenson, C. M., Haoa, S., Mulrooney, M., Puleston, C. O., Vitousek, P. M., & Chadwick, O. A. (2010). Soil nutrient analysis of Rapa Nui gardening. Archaeology In Oceania/Archæology & Physical Anthropology In Oceania, 45(2), 80-85. https://doi.org/10.1002/j.1834-4453.2010.tb00082.x

Lepetit, M., & Brouquisse, R. (2023). New insights into the control of symbiotic nitrogen fixation by carbon supply. *Molecular Plant*, *16*(11), 1724-1726. https://doi.org/10.1016/j.molp.2023.09.021

Li, F. L., Bao, W. K., & Wu, N. (2009). Effects of water stress on growth, dry matter allocation and water-use efficiency of a leguminous species, *Sophora davidii*. *Agroforestry systems*, *77*, 193-201. https://doi.org/10.1007/s10457-008-9199-1

Liao, Z., Fan, J., Lai, Z., Bai, Z., Wang, H., Cheng, M., Zhang, F., & Li, Z. (2023). Response network and regulatory measures of plant-soil-rhizosphere environment to drought stress. In Advances in agronomy (pp. 93-196). https://doi.org/10.1016/bs.agron.2023.03.002

Ligero, F., Lluch, C., & Olivares, J. (1986). Evolution of ethylene from roots of Medicago sativa plants inoculated with *Rhizobium meliloti*. Journal of plant physiology, 125(3-4), 361-365. https://doi.org/10.1016/S0176-1617(86)80158-4

Ljung K. (2013). Auxin metabolism and homeostasis during plant development. Development (Cambridge, England), 140(5), 943–950. https://doi.org/10.1242/dev.086363

Lohr, D., Tillmann, P., Druege, U., Zerche, S., Rath, T., & Meinken, E. (2017). Non-destructive determination of carbohydrate reserves in leaves of ornamental cuttings by near-infrared spectroscopy (NIRS) as a key indicator for quality assessments. Biosystems Engineering, 158, 51-63. https://doi.org/10.1016/j.biosystemseng.2017.03.005

Ma, W., Penrose, D. M., & Glick, B. R. (2002). Strategies used by rhizobia to lower plant ethylene levels and increase nodulation. Canadian journal of microbiology, *48*(11), 947-954. https://doi.org/10.1139/w02-100

Maltz, M. R., & Treseder, K. K. (2015). Sources of inocula influence mycorrhizal colonization of plants in restoration projects: A meta-analysis. Restoration Ecology, **23**, 625–634. https://doi.org/10.1111/rec.12231

Mansour, A. (2009). Phylip and phylogenetics. Focus on Bioinformatics: Genes, Genomes and Genomics, 3, 46-49.

Marino, D., Frendo, P., Ladrera, R., Zabalza, A., Puppo, A., Arrese-Igor, C., & González, E. M. (2007). Nitrogen fixation control under drought stress. Localized or systemic?. Plant physiology, *143*(4), 1968–1974. https://doi.org/10.1104/pp.106.097139

Marques, A. S., Marchaison, A., Gardan, L., y Samson, R. (2008). BOX-PCR-based identification of bacterial species belonging to *Pseudomonas syringae*: *P. viridiflava group.* Genet. Mol. Biol., 31, 106-115. https://doi.org/10.1590/S1415-47572008000100019

Mathesius, U. (2020). Hormonal interactions in the regulation of the nitrogen-fixing legume-Rhizobium symbiosis. In Advances in Botanical Research (Vol. 94, pp. 41-66). Academic Press. https://doi.org/10.1016/bs.abr.2019.09.010

Maunder, M., Culham, A., Alden, B., Zizka, G., Orliac, C., Lobin, W., Bordeu, A., Ramirez, J. M., y Glissmann-Gough, S. 2000. Conservation of the Toromiro Tree: Case Study in the Management of a Plant Extinct in the Wild. Conserv. Biol., 14(5), 1341–1350. http://www.jstor.org/stable/2641783

Meena, V. S., Meena, S. K., Verma, J. P., Kumar, A., Aeron, A., Mishra, P. K., Bisht, J. K., Pattanayak, A., Naveed, M., & Dotaniya, M. (2017). Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: A review. Ecological Engineering, 107, 8-32. https://doi.org/10.1016/j.ecoleng.2017.06.058

Mieth, A., & Bork, H. (2005). History, origin and extent of soil erosion on Easter Island (Rapa Nui). Catena (Cremlingen), 63(2-3), 244-260. https://doi.org/10.1016/j.catena.2005.06.011

Mikiciuk, G., Miller, T., Kisiel, A., Cembrowska-Lech, D., Mikiciuk, M., Łobodzińska, A., & Bokszczanin, K. (2024). Harnessing Beneficial Microbes for Drought Tolerance: A Review of Ecological and Agricultural Innovations. Agriculture, 14(12), 2228. https://doi.org/10.3390/agriculture14122228

Mujumdar, S., Bhoyar, J., Akkar, A., Hundekar, S., Agnihotri, N., Jaybhay, P., & Bhuyan, S. (2023). Acinetobacter: A versatile plant growth-promoting rhizobacteria (PGPR). In Elsevier eBooks (pp. 327-362). https://doi.org/10.1016/b978-0-323-91875-6.00009-8

Nagargade, M., Tyagi, V., Singh, M.K. (2018). Plant Growth-Promoting Rhizobacteria: A Biological Approach Toward the Production of Sustainable Agriculture. In: Meena, V. (eds) Role of Rhizospheric Microbes in Soil. Springer, Singapore. https://doi.org/10.1007/978-981-10-8402-7_8

Nascimento, F. X., Brígido, C., Glick, B. R., & Rossi, M. J. (2016). The role of rhizobial ACC deaminase in the nodulation process of leguminous plants. International Journal of Agronomy, 2016(1), 1369472. https://doi.org/10.1155/2016/1369472

Nazir, F., Peter, P., Gupta, R., Kumari, S., Nawaz, K., & Khan, M. I. R. (2024). Plant hormone ethylene: A leading edge in conferring drought stress tolerance. Physiologia Plantarum, 176(1), e14151. https://doi.org/10.1111/ppl.14151

Nwigwe, C. S. (2018). Root-enhancing properties of rhizospheric bacteria on Eucalyptus hybrid cuttings (Doctoral dissertation, Bloemfontein: Central University of Technology, Free State).

Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. World journal of microbiology & biotechnology, 33(11), 197. https://doi.org/10.1007/s11274-017-2364-9

Olave, I. (2020). Identificación y selección de rizobios para *Sophora toromiro* (Phil) Skottsb., especie extinta en Rapa Nui. Tesis Ingeniero Agrónomo. Universidad de Concepción. 34 pp.

Parewa, H.P., Meena, V.S., Jain, L.K., Choudhary, A. (2018). Sustainable Crop Production and Soil Health Management Through Plant Growth-Promoting Rhizobacteria. In: Meena, V. (eds) Role of Rhizospheric Microbes in Soil. Springer, Singapore. https://doi.org/10.1007/978-981-10-8402-7_12

Pawson, S. M., Brin, A., Brockerhoff, E. G., Lamb, D., Payn, T. W., Paquette, A., & Parrotta, J. A. (2013). Plantation forests, climate change and biodiversity. Biodiversity and Conservation, *22*, 1203-1227. https://doi.org/10.1007/s10531-013-0458-8

Penrose, D. and B. Glick. (2003). Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol. Plant., 118: 10-15

Pezoa, I., Villacreses, J., Rubilar, M., Pizarro, C., Galleguillos, M. J., Ejsmentewicz, T., Fonseca, B., Espejo, J., Polanco, V., & Sánchez, C. (2021). Generation of Chloroplast Molecular Markers to Differentiate Sophora toromiro and Its Hybrids as a First Approach to Its Reintroduction in Rapa Nui (Easter Island). Plants, 10(2), 342. https://doi.org/10.3390/plants10020342

PHOLO-TAIT, M., KGETSE, T., TSHEKO, G. N., THEDI, O. T., LETHOLA, K., MOTLAMME, E. O., ITHUTENG, M. I., & NGWAKO, S. (2022). Genotypic variation in response to drought stress is associated with biochemical and transcriptional regulation of ureides metabolism in common bean (*Phaseolus vulgaris* L.). Acta Agriculturae Slovenica, *118*(2), 1–9. https://doi.org/10.14720/aas.2022.118.2.2541.

Premono, M. E., Moawad, A. M., & Vlek, P. L. G. (1996). Effect of phosphate-solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. (No. REP-12113. CIMMYT.).

Püschel, T. A., Espejo, J., Sanzana, M., & Benítez, H. A. (2014). Analysing the Floral Elements of the Lost Tree of Easter Island: A Morphometric Comparison between the Remaining Ex-Situ Lines of the Endemic Extinct Species *Sophora toromiro*. PloS One, 9(12), e115548. https://doi.org/10.1371/journal.pone.0115548

Ranawat, B., Mishra, S., & Singh, A. (2021). *Enterobacter hormaechei* (MF957335) enhanced yield, disease and salinity tolerance in tomato. *Archives of microbiology*, *203*(5), 2659–2667. https://doi.org/10.1007/s00203-021-02226-5.

Rashid, S., T. Charles and B. Glick. (2012). Isolation and characterization of new plant growth-promoting bacterial endophytes. Appl. Soil ecology. 61: 217-224. https://doi.org/10.1016/j.apsoil.2011.09.011

Roslan, M. A. M., Zulkifli, N. N., Sobri, Z. M., Zuan, A. T. K., Cheak, S. C., & Rahman, N. A. A. (2020). Seed biopriming with P- and K-solubilizing *Enterobacter hormaechei* sp. improves the early vegetative growth and the P and K uptake of okra (*Abelmoschus esculentus*) seedling. PLoS ONE, 15(7), e0232860. https://doi.org/10.1371/journal.pone.0232860

Sánchez, D., & Bonilla, R. (2014). Respuesta vegetal de acacia decurrens a la inoculación con rizobacterias promotoras de crecimiento vegetal bajo estrés salino. *Temas Agrarios*, *19*(2), 159-172. https://doi.org/10.21897/rta.v19i2.731

Sarwar M, Kremer RJ. (1995). Determination of bacterially derived auxins using a microplate method. Lett Appl Microbiol 20:282–28. https://doi.org/10.1111/j.1472-765X.1995.tb00446.x

Shams, E., Etemadi, N., and M. Nezhad. (2015). Effects of indole butyric acid on rooting of hardwood cuttings of *Sophora japonica* L. J. Sci. & Technol. Greenhouse Culture 6 (23): 63- 77

Shome, S., Barman, A., & Solaiman, Z. M. (2022). Rhizobium and phosphate solubilizing bacteria influence the soil nutrient availability, growth, yield, and quality of soybean. *Agriculture*, *12*(8), 1136. https://doi.org/10.3390/agriculture12081136

Simonsen, A.K., Dinnage, R., Barrett, L.G., Prober, S.M. & Thrall, P.H. 2017. Symbiosis limits establishment of legumes outside their native range at a global scale. Nature Communications 8: 14790. https://doi.org/10.1038/ncomms14790

Skottsberg, C. (1920). The Phanerograms of Easter Island. Pages 61–84, 487–502 in C. Skottsberg, editor. The natural history of Juan Fernandez and Easter Island. Volume 2. Almqvist & Wiksells Boktryckeri Ab., Uppsala, Sweden.

Souza, J. M. A., Leonel, S., Silva, M. de S., Oliveira Júnior, M. de A., Martins, R. C., Bolfarini, A. C. B., & Ataíde, E. M. (2019). Carbohydrate content and season collection of cuttings from 'Roxo de Valinhos' fig tree. Comunicata Scientiae, *10*(1), 125–131. https://doi.org/10.14295/cs.v10i1.2902

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution 28: 2731-2739 https://doi.org/10.1093/molbev/msr121

Toju, H., Peay, K. G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., Onoda, Y., Yoshida, K., Schlaeppi, K., Bai, Y., Sugiura, R., Ichihashi, Y., Minamisawa, K., & Kiers, E. T. (2018). Core microbiomes for sustainable agroecosystems. Nature Plants, 4(5), 247-257. https://doi.org/10.1038/s41477-018-0139-4

Tsafouros, A., Frantzeskaki, A., Assimakopoulou, A., & Roussos, P. A. (2019). Spatial and temporal changes of mineral nutrients and carbohydrates in cuttings of four stone fruit rootstocks and their contribution to rooting potential. Scientia Horticulturae, 253, 227-240. https://doi.org/10.1016/j.scienta.2019.04.049

Valliere, J. M., Zhang, J., Sharifi, M. R., & Rundel, P. W. (2019). Can we condition native plants to increase drought tolerance and improve restoration success?. Ecological applications: a publication of the Ecological Society of America, *29*(3), e01863. https://doi.org/10.1002/eap.1863.

Valliere, J. M., Wong, W. S., Nevill, P. G., Zhong, H., & Dixon, K. W. (2020). Preparing for the worst: Utilizing stress-tolerant soil microbial communities to aid ecological restoration in the Anthropocene. Ecological Solutions and Evidence, 1(2), e12027. https://doi.org/10.1002/2688-8319.12027

Van de Poel, B., and Van Der Straeten, D. (2014). 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: more than just the precursor of ethylene! Front. Plant Sci. 5:640. doi: 10.3389/fpls.2014.00640

Vásquez, S. C., Molina-Müller, M. L., Uquillas, C. A. M., Fahrenkrog, P. S., & Sánchez, E. I. V. (2020). In vitro organogenesis in Sophora toromiro from nodal segments.

Vekemans, X., Beauwens, T., Lemaire, M., & Roldán-Ruiz, I. (2002). Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. Molecular ecology, *11*(1), 139–151. https://doi.org/10.1046/j.0962-1083.2001.01415.x.

Velázquez, E., Carro, L., Flores-Félix, J.D., Menéndez, E., Ramírez-Bahena, MH., Peix, A. (2019). Bacteria-Inducing Legume Nodules Involved in the Improvement of Plant Growth, Health and Nutrition. In: Kumar, V., Prasad, R., Kumar, M., Choudhary, D. (eds) Microbiome in Plant Health and Disease. Springer, Singapore. https://doi.org/10.1007/978-981-13-8495-0_4

Verma, R., Annapragada, H., Katiyar, N., Shrutika, N., Das, K., & Senthilkumar, M. (2020). Chapter 4 - Rhizobium. En Beneficial Microbes in Agro-Ecology (pp. 37-54). https://doi.org/10.1016/b978-0-12-823414-3.00004-6

Versalovic, J., Schneider, M., De Bruijn, F. J., y Lupski, J. R. (1994). Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods in Molecular and Cellular Biology, 5(1), 25-40

Vishwakarma K, Kumar N, Shandilya C, Mohapatra S, Bhayana S and Varma A. (2020). Revisiting Plant–Microbe Interactions and Microbial Consortia

Application for Enhancing Sustainable Agriculture: A Review. Front. Microbiol. 11:560406. doi: 10.3389/fmicb.2020.560406

Wang, Y., Liu, H., Shen, Z., Yu, M., Wang, J., Jiang, X., & Shen, Q. (2022). Richness and antagonistic effects co-affect plant growth promotion by synthetic microbial consortia. Applied Soil Ecology, 170, 104300. https://doi.org/10.1016/j.apsoil.2021.104300

Wang, J., Deng, Z., Gao, X., Long, J., Wang, Y., Wang, W., Li, C., He, Y., & Wu, Z. (2024). Combined control of plant diseases by Bacillus subtilis SL44 and Enterobacter hormaechei Wu15. The Science Of The Total Environment, 934, 173297. https://doi.org/10.1016/j.scitotenv.2024.173297

Wasilewski, M. (2012). Prehistory of Rapa Nui (Easter Island): a general outline.

Witt, T. W., Northup, B. K., Porch, T. G., Barrera, S., & Urrea, C. A. (2023). Effect of cutting management on the forage production and quality of tepary bean (*Phaseolus acutifolius* A. Gray). Scientific Reports, *13*(1), 12875. https://doi.org/10.1038/s41598-023-39550-3

Woo, S. L., & Pepe, O. (2018). Microbial Consortia: Promising Probiotics as Plant Biostimulants for Sustainable Agriculture. Frontiers In Plant Science, 9. https://doi.org/10.3389/fpls.2018.01801

Wu, F., Bao, W., Li, F., & Wu, N. (2008). Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of *Sophora davidii* seedlings. Environmental and experimental botany, 63(1-3), 248-255. https://doi.org/10.1016/j.envexpbot.2007.11.002

Yadav, D., & Singh, S. P. (2018). Vegetative methods of plant propagation: I-cutting layering and budding. Journal of Pharmacognosy and Phytochemistry, *7*(2), 3267-3273.

Yates, R. J., Howieson, J. G., Hungria, M., Bala, A., O'Hara, G. W., & Terpolilli, J. (2016). Authentication of rhizobia and assessment of the legume symbiosis in controlled plant growth systems (Ill.). In J. G. Howieson & M. J. Dilworth (Editors), *Working with rhizobia* (pp. 73–108). Australian Centre for International Agricultural Research. https://researchportal.murdoch.edu.au/esploro/outputs/bookChapter/Authentication-of-rhizobia-and-assessment-of/991005543590107891#file-0

Yeremko, L., Czopek, K., Staniak, M., Marenych, M., & Hanhur, V. (2025). Role of Environmental Factors in Legume-*Rhizobium* Symbiosis: A Review. Biomolecules, *15*(1), 118. https://doi.org/10.3390/biom15010118

Zaidi, A., Khan, M.S., Rizvi, A., Saif, S., Ahmad, B., Shahid, M. (2017). Role of Phosphate-Solubilizing Bacteria in Legume Improvement. In: Zaidi, A., Khan, M., Musarrat, J. (eds) Microbes for Legume Improvement. Springer, Cham. https://doi.org/10.1007/978-3-319-59174-2_8

Zhao, X., Yuan, X., Xing, Y., Dao, J., Zhao, D., Li, Y., ... & Wang, Z. (2023). A meta-analysis on morphological, physiological and biochemical responses of plants with PGPR inoculation under drought stress. Plant, Cell & Environment, 46(1), 199-214. https://doi.org/10.1111/pce.14466

Zhu, H., Liu, X., Zhang, F., & Yao, Q. (2019). Chapter 2 - Promoting Crop Growth With Symbiotic Microbes in Agro-Ecosystems in Climate Change Era. In New and Future Developments in Microbial Biotechnology and Bioengineering (pp. 31-41). https://doi.org/10.1016/b978-0-444-64191-5.00002-x

Zizka, G., Zizka, A. (2022). The Flora and Vegetation of Easter Island: Past and Present. In: Rull, V., Stevenson, C. (eds) The Prehistory of Rapa Nui (Easter Island). Developments in Paleoenvironmental Research, vol 22. Springer, Cham.

Zulfitri, A. (2012). Plant growth promotion by IAA-producing rhizobacteria in ornamental plant propagation (Master's thesis, University of Sydney).

CAPÍTULO 3

CONCLUSIONES GENERALES

Este estudio identificó bacterias nativas con capacidad de promover el crecimiento vegetal, presentes en suelos vinculados a *Sophora spp.* y en distintos sitios de Rapa Nui. La aplicación de consorcios bacterianos compuestos por PGPR y rizobios nativos en *S. toromiro* evidenció un efecto positivo en la formación de nódulos y en el sistema radicular, especialmente bajo condiciones de sequía. El empleo de estacas como material de propagación conlleva limitaciones fisiológicas que podrían afectar la simbiosis planta-microorganismo, debido a sus bajas reservas energéticas y al lento desarrollo inicial del sistema radicular, lo que posiblemente redujo la respuesta temprana a la inoculación.

La duración de los ensayos representa un elemento clave al analizar interacciones planta-microbio en especies de desarrollo lento como *S. toromiro*. Futuros estudios deberían considerar ensayos de mayor duración para profundizar en los efectos de la inoculación microbiana en *S. toromiro* y otras especies con características similares.

No obstante, la incorporación de consorcios microbianos funcionales y adaptados localmente se perfila como una herramienta valiosa para apoyar y favorecer la reintroducción ecológica de *Sophora toromiro* en su entorno natural.