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EFECTOS DE EXTRACTOS DE ALGAS *MACROCYSTIS PYRIFERA* COMBINADOS CON *PSEUDOMONAS KOREENSIS* SOBRE EL CRECIMIENTO Y LA RESPUESTA ESTOMÁTICA AL ESTADO HÍDRICO EN PORTAINJERTOS 'COLT' DE CEREZO DULCE

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CARMEN MACARENA CRUZAT HERMOSILLA
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Profesor Guía: Arturo Calderón Orellana
Dpto. de Producción Vegetal
Facultad de Agronomía
Universidad de Concepción

Esta tesis ha sido realizada en el Departamento de Producción Vegetal de la Facultad de Agronomía, Universidad de Concepción.

Profesor Guía

Dr. Arturo Calderón Orellana
Facultad de Agronomía
Universidad de Concepción

Profesor Co-Guía

Dra. Macarena Gerding González
Facultad de Agronomía
Universidad de Concepción

Comisión Evaluadora:

Dr. Miguel Garriga Caraballo
Facultad de Agronomía
Universidad de Concepción

Dr. Ignacio Serra Stepke
Facultad de Agronomía
Universidad de Concepción

Dr. Lorena Barra Bucarei
CEO – Encargada I+D+i MacBio

Director (s) de Programa

Dra. Claudia Muñoz Espinoza
Facultad de Agronomía
Universidad de Concepción

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RESUMEN

El portainjerto Colt (*Prunus avium* × *Prunus pseudocerasus*) se utiliza ampliamente en los huertos de cerezos chilenos debido a su alta compatibilidad y vigor; sin embargo, su baja tolerancia a la sequía limita su rendimiento en entornos con escasez de agua. Los bioestimulantes derivados de extractos de algas marinas combinados con bacterias promotoras del crecimiento vegetal (PGPB) han demostrado efectos positivos en cultivos herbáceos, pero su impacto en especies leñosas sigue sin estar muy estudiado. Este estudio evaluó las respuestas fisiológicas y morfológicas de plantas jóvenes de Colt a la aplicación de extractos de *Macrocystis pyrifera* obtenidos por hidrólisis alcalina (A2) e hidrólisis mixta (alcalina + enzimática, A1), solos o combinados con *Pseudomonas koreensis* (AG-97), bajo riego abundante (WET) y riego deficitario (DRY). En condiciones WET, el tratamiento A1B aumentó la conductancia estomática en un 90 % y redujo la temperatura de las hojas (~2 °C), mitigando el riesgo de hipoxia durante los periodos de alta humedad del sustrato. Estas mejoras se asociaron con una mayor eficiencia fotoquímica (*Fv*/*Fm*) en condiciones de baja radiación y alargamiento del tallo (+23 cm frente a 10 cm en el control). En condiciones DRY, A1B redujo la conductancia estomática en ~70 % con un potencial hídrico < –1,1 MPa, lo que limitó la deshidratación y redujo la defoliación a menos del 15 % en comparación con el 40 % en las plantas tratadas solo con tratamiento B. Los tratamientos A1 y A1B también mostraron una mayor asimilación de carbono (>41 %). Estos resultados confirman un efecto sinérgico entre los extractos de algas marinas y las PGPB, mejorando la resiliencia fisiológica y el crecimiento del portainjerto Colt en regímenes hídricos contrastados.

SUMMARY

The Colt rootstock (*Prunus avium* × *Prunus pseudocerasus*) is widely used in Chilean cherry orchards due to its high compatibility and vigor; however, its low drought tolerance limits its performance in water-scarce environments. Biostimulants derived from seaweed extracts combined with plant growth-promoting bacteria (PGPB) have shown positive effects on herbaceous crops, but their impact on woody species remains largely unexplored. This study evaluated the physiological and morphological responses of young Colt plants to the application of *Macrocystis pyrifera* extracts obtained by alkaline hydrolysis (A2) and mixed hydrolysis (alkaline + enzymatic, A1), alone or combined with *Pseudomonas koreensis* (AG-97), under abundant irrigation (WET) and deficit irrigation (DRY). Under WET conditions, treatment A1B increased stomatal conductance by 90% and reduced leaf temperature (~2 °C), mitigating the risk of hypoxia during periods of high substrate moisture. These improvements were associated with greater photochemical efficiency (*Fv*/*Fm*) under low radiation conditions and stem elongation (+23 cm vs. 10 cm in the control). Under DRY conditions, A1B reduced stomatal conductance by ~70% with water potential < –1.1 MPa, limiting dehydration and reducing defoliation to less than 15% compared to 40% in plants treated with PGPB alone. Treatments A1 and A1B also showed higher carbon assimilation (>41%). These results confirm a synergistic effect between seaweed extracts and PGPB, improving the physiological resilience and growth of the Colt rootstock under contrasting water regimes.

CAPÍTULO 1. INTRODUCCIÓN GENERAL

La fruticultura en Chile ha experimentado un crecimiento sostenido durante los últimos tres años, con una tasa promedio anual de 3,1%, alcanzando una superficie plantada de 386.573 hectáreas. Este desarrollo ha sido impulsado principalmente por especies de alto valor comercial, como el cerezo (*Prunus avium* L.). El aumento en la superficie establecida se ha concentrado en las zonas centro-sur del país, reflejando un desplazamiento territorial de la producción frutícola (ODEPA, 2025). Este cambio responde a los crecientes desafíos agroclimáticos que enfrenta la zona central, históricamente la principal área productiva, la cual actualmente representa el 77% de la superficie frutícola nacional (ODEPA, 2025).

En esta región se ha registrado una disminución progresiva de las precipitaciones invernales, con una caída estimada entre un 20% y un 30% en la última década (MMA, 2024). Además, los modelos climáticos proyectan un aumento en el número de días secos, intensificando las condiciones de sequía. Este fenómeno se asocia a un incremento en la temperatura media anual (+4,1°C), cambios en los patrones de precipitación y una reducción en las reservas de agua dulce (-39%) (Bambach *et al*., 2021), configurando un escenario desafiante para la sostenibilidad de la fruticultura nacional. Estas condiciones han afectado la disponibilidad de agua para riego, incentivando la búsqueda de estrategias de mitigación frente al cambio climático.

La reducción en la disponibilidad hídrica en los huertos frutales puede generar estrés hídrico moderado a severo durante etapas críticas del ciclo productivo (Santibáñez *et al*., 2013). Esta condición puede provocar alteraciones significativas en los procesos fisiológicos y morfológicos de las plantas, como disminución del intercambio gaseoso, limitación de la fotosíntesis y reducción en la producción de biomasa (Moreno, 2009). En los agroecosistemas frutales, el impacto del estrés hídrico depende tanto del momento en que ocurre como de su duración. Un episodio severo durante una fase crítica de desarrollo, especialmente si es prolongado, puede afectar gravemente el crecimiento vegetativo y el rendimiento productivo (Morales *et al*., 2013). En el caso del cerezo, se ha observado que un estrés hídrico severo en el periodo pre-cosecha puede reducir el peso de poda hasta en un 40%, evidenciando su impacto directo en el vigor y la productividad de la planta (Blanco *et al*., 2018). Este tipo de estrés reduce drásticamente el crecimiento de brotes y hojas, especialmente en cerezos jóvenes, debido a la disminución de la división y elongación celular en los tejidos meristemáticos (Catalán-Paine *et al*., 2025). Además, se altera el metabolismo de azúcares en las hojas, con una disminución en la concentración de sorbitol (41%) y fructosa (35%), afectando el metabolismo energético de la planta (Blaya-Ros *et al*., 2020).

La respuesta de las plantas al estrés hídrico está directamente relacionada con las características fisiológicas y morfológicas del portainjerto. Bajo condiciones de sequía, el crecimiento radicular y la solubilidad de nutrientes disminuyen, afectando negativamente su absorción y transporte dentro de la planta (Gunes *et al*., 2006). Por ello, los portainjertos cumplen un papel crucial en la tolerancia al déficit hídrico, ya que su capacidad para explorar el perfil del suelo y aprovechar el agua disponible depende de su estructura y densidad radicular. Aquellos considerados tolerantes presentan sistemas radiculares más profundos y eficientes, lo que les permite mantener la funcionalidad hídrica y nutricional bajo condiciones de sequía (Romero *et al*., 2006).

Entre los portainjertos más utilizados en Chile se encuentra Colt (*Prunus avium* × *Prunus pseudocerasus*), comúnmente empleado en el cultivo de cerezo dulce por su alta compatibilidad con la mayoría de las variedades comerciales y su vigor medio-alto (~80% en comparación con el portainjerto F12/1). Sin embargo, su sistema radicular superficial lo hace sensible a la sequía, limitando su desempeño bajo condiciones de déficit hídrico. A pesar de ello, Colt presenta una notable tolerancia al mal drenaje y a la asfixia radicular, lo que lo convierte en una opción adecuada para suelos pesados con buena disponibilidad de agua (Pino *et al*., 2014; Catalán-Paine e*t al*., 2025).

Diversos estudios han demostrado que, bajo condiciones de déficit hídrico, los cerezos injertados sobre Colt presentan una reducción significativa en el crecimiento vegetativo y productivo, caracterizada por menor área foliar, menor elongación de brotes, frutos de menor tamaño y rendimientos proporcionales a la severidad del estrés (Blaya-Ros *et al*., 2020). En contraste, portainjertos como Mahaleb y Afgano, considerados más tolerantes a la sequía (Pino *et al*., 2014), mantienen mayores tasas de fotosíntesis y conductancia estomática bajo condiciones de escasez hídrica, lo que contribuye a preservar su actividad fisiológica y rendimiento.

A pesar de estas limitaciones, el portainjerto Colt ha sido ampliamente adoptado en la fruticultura moderna en Chile. Desde 2004, se ha registrado un aumento sostenido en la venta de plantas injertadas sobre Colt, consolidando su posición como el portainjerto más plantado en el país en los últimos años. Entre 2008 y 2013, representó consistentemente más de la mitad de los cerezos vendidos anualmente en viveros, alcanzando entre un 50% y un 61% durante este período (AGV, 2014). Su uso se ha concentrado especialmente en zonas con suelos de buena retención hídrica, debido a su alta compatibilidad con variedades productivas y su resistencia al mal drenaje y a la asfixia radicular (Ayala, 2009).

Ante escenarios de inestabilidad climática y baja disponibilidad hídrica, la eficiencia en el uso del agua se ha convertido en una prioridad para la agricultura. La producción de biomasa está vinculada a la cantidad de agua disponible en el suelo, y se estima que entre el 50% y el 80% del agua accesible se utiliza en actividades agrícolas (Medrano *et al*., 2007). En este contexto, el uso de bioestimulantes y microorganismos benéficos surge como una estrategia prometedora para mejorar la resiliencia de los cultivos,

Los bioestimulantes derivados de algas, especialmente algas pardas como *Macrocystis pyrifera*, han demostrado mejorar la eficiencia en el uso del agua, el desempeño fotosintético y la defensa antioxidante en las plantas. Su efectividad depende del método de extracción, siendo la hidrólisis mixta (alcalina + enzimática) la más eficiente (Thevanthan *et al*., 2005). Por otro lado, las PGPB, como *Pseudomonas koreensis*, contribuyen a la tolerancia al estrés hídrico mediante la producción de fitohormonas, osmolitos y enzimas, además de mejorar la retención de humedad y la absorción de nutrientes (Sati *et al*., 2021).

Aunque la aplicación sinérgica de extractos de algas marinas y PGPB ha mostrado resultados positivos en cultivos herbáceos, sus efectos en especies frutales leñosas perennes, como los portainjertos de cerezo, permanecen inexplorados. Thevathan *et al.* (2005), sugieren que esta combinación puede activar redes de señalización complejas que mejoran la resiliencia de las plantas al déficit hídrico. Estudios recientes en palmeras tropicales confirman este potencial, demostrando que los bioestimulantes combinados mejoran el intercambio gaseoso, el ajuste osmótico y la actividad antioxidante en condiciones de sequía (Rodrigues *et al*., 2025). El uso de estas estrategias es particularmente relevante en Chile, especialmente para portainjertos como Colt, que se utilizan ampliamente en huertos tradicionales y se caracterizan por su baja tolerancia a la sequía. En este contexto, el presente estudio tuvo como objetivo evaluar el efecto de la aplicación de extractos de *Macrocystis pyrifera*, obtenidos mediante hidrólisis alcalina y mixta (alcalina + enzimática), en combinación con la bacteria PGPB *Pseudomonas koreensis* AG-97, en plantas jóvenes de portainjerto Colt. La evaluación se realizó bajo condiciones de riego abundante y déficit hídrico, analizando sus respuestas fisiológicas y morfológicas frente a diferentes niveles de disponibilidad de agua.

HIPÓTESIS

La aplicación combinada de extractos de *Macrocystis pyrifera* (obtenidos por hidrólisis alcalina y mixta) y la bacteria promotora del crecimientovegetal *Pseudomonas koreensis* AG-97 mejora la tolerancia al déficit hídrico y la eficiencia fisiológica del portainjerto Colt.

OBJETIVO GENERAL

Evaluar el efecto de extractos de *Macrocystis pyrifera*, obtenidos por dos métodos de extracción (hidrólisis alcalina y mixta), aplicados solos o en combinación con *Pseudomonas koreensis* AG-97, sobre las respuestas fisiológicas y morfológicas del portainjerto Colt (*Prunus avium* × *Prunus pseudocerasus*) bajo condiciones de riego abundante y déficit hídrico.

OBJETIVOS ESPECÍFICOS

1. Verificar la compatibilidad entre los extractos de *Macrocystis pyrifera* y la cepa de *Pseudomonas koreensis*, así como su persistencia en la rizósfera del portainjerto

2. Caracterizar la respuesta hídrica de los tratamientos del portainjerto Colt, mediante la medición del potencial hídrico del tallo y la dinámica de humedad del sustrato bajo dos regímenes de riego: abundante (WET) y deficitario (DRY).

3. Determinar el efecto de los tratamientos sobre la regulación estomática y la eficiencia fotosintética, evaluando variables como la conductancia estomática, la temperatura foliar y la eficiencia del fotosistema II (*Fv/Fm*).

4. Analizar la influencia de los tratamientos en el crecimiento vegetativo y la distribución de biomasa, cuantificando la elongación del tallo, el número de hojas y el peso seco de raíces, tallo y hojas.

5. Evaluar los tratamientos la eficiencia en el uso del agua y la asimilación de carbono mediante la determinación del contenido de carbono y la discriminación isotópica ($\delta^{13}C$) en hojas.

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CAPÍTULO 2.

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Macrocystis pyrifera seaweed extracts combined with *Pseudomonas koreensis* stimulate growth and change stomatal response to contrasting plant water status in sweet cherry rootstock Colt

Macarena Cruzat-Hermosilla[1,2], Tamara Alvear[1], Arturo Calderón-Orellana[1*], Macarena Gerding[1], Marcelo Brintrup[2] and Miguel Garriga[1]

[1]Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Concepción, Chillán 3780000, Chile

[2]Patagonia Biotecnología S.p.A, Puerto Montt 5400000, Chile

*** Correspondence:**
Arturo Calderón-Orellana
arcalderon@udec.cl

Keywords: *Prunus avium* L., Colt rootstock, Seaweed extracts, *Macrocystis pyrifera*, *Pseudomonas koreensis*, Plant growth-promoting bacteria, Drought tolerance, Stomatal conductance, Photosystem II efficiency.

Abstract

The Colt rootstock (*Prunus avium* × *Prunus pseudocerasus*) is widely used in Chilean cherry orchards due to its high compatibility and vigor; however, its low drought tolerance limits its performance in water-scarce environments. Biostimulants derived from seaweed extracts combined with plant growth-promoting bacteria (PGPB) have shown positive effects on herbaceous crops, but their impact on woody species remains largely unexplored. This study evaluated the physiological and morphological responses of young Colt plants to the application of *Macrocystis pyrifera* extracts obtained by alkaline hydrolysis (A1) and mixed hydrolysis (alkaline + enzymatic; A2), applied alone or combined with *Pseudomonas koreensis* AG-97 (B). Treatment combinations included A1B (A1 + *P. koreensis* AG-97), A2B (A2 + *P. koreensis* AG-97), and B (bacteria alone). Under well-watered conditions (WET), which led to periods of substrate saturation conducive to transient root-zone hypoxia, treatment A1B increased stomatal conductance by 90% and reduced leaf temperature (~2 °C), helping mitigate the physiological stress associated with excess moisture. These improvements were associated with greater photochemical efficiency (*Fv*/*Fm*) under low-light conditions and enhanced shoot elongation (+23 cm vs. 10 cm in the control). Under deficit irrigation (DRY), A1B reduced stomatal conductance by ~70% when plant water potential dropped below –1.1 MPa, limiting dehydration and reducing defoliation to less than 15%, compared to 40% in plants treated with B alone. Treatments A1 and A1B also showed higher carbon assimilation (>41%). These results confirm a synergistic effect between seaweed extracts and PGPB, improving the physiological resilience and growth of the Colt rootstock under contrasting water regimes.

1 Introduction

Fruit production in Chile has grown steadily over the past three years, with an average annual rate of 3.1%, reaching a planted area of 386,573 hectares. This development has been driven primarily by high-value species, such as cherry (*Prunus avium* L.). The increase in the established area has been concentrated in the south-central regions of the country, reflecting a territorial shift in fruit production (ODEPA, 2025). This change responds to the growing agroclimatic challenges facing the central zone, historically the main productive area, which currently accounts for 77% of the national fruit-growing area (ODEPA, 2025).

In this region, winter rainfall has declined by an estimated 20%-30% over the last decade (MMA, 2024). In addition, climate models project an increase in the number of dry days, intensifying drought conditions. This phenomenon is associated with an increase in the average annual temperature (+4.1°C), changes in precipitation patterns, and a reduction in freshwater reserves (-39%) (Bambach *et al*., 2021), creating a challenging scenario for the sustainability of national fruit growing. These conditions have affected the availability of water for irrigation, encouraging the search for climate change mitigation strategies.

Reduced water availability in fruit orchards can lead to moderate to severe water stress during critical stages of the production cycle (Santibáñez *et al*., 2013). This condition can cause significant alterations in plant physiological and morphological processes, including reduced gas exchange, limited photosynthesis, and decreased biomass production (Moreno, 2009). In fruit agroecosystems, the impact of water stress depends on both the timing and duration of stress. A severe episode during a critical developmental phase, especially if prolonged, can significantly affect vegetative growth and yield (Morales *et al*., 2013). In cherry trees, severe water stress during the pre-harvest period can reduce pruning weight by up to 40%, indicating a direct effect on plant vigor and productivity (Blanco *et al*., 2018). This type of stress markedly reduces shoot and leaf growth, particularly in young cherry trees, due to reduced cell division and elongation in meristematic tissues (Catalán-Paine *et al*., 2025). In addition, there is an alteration in leaf sugar metabolism, with decreases in sorbitol (41%) and fructose (35%) concentrations, which affects plant energy metabolism (Blaya-Ros *et al*., 2020).

The response of plants to water stress is directly related to the physiological and morphological characteristics of the rootstock. Under drought conditions, root growth and nutrient solubility decrease, thereby reducing their absorption and transport within the plant (Gunes *et al*., 2006). This is why rootstocks play a crucial role in water deficit tolerance, as their ability to explore the soil profile and utilize available water depends on their structure and root density. Those considered tolerant have deeper and more efficient root systems, which enable them to maintain water and nutrient uptake under drought conditions (Romero *et al*., 2006).

Among the most widely used rootstocks in Chile is Colt (*Prunus avium* × *Prunus pseudocerasus*), commonly used in sweet cherry cultivation due to its high compatibility with most commercial varieties and its medium-high vigor (~ 80% compared to the F12/1 rootstock). However, its shallow root system makes it sensitive to drought, limiting its performance under water deficit conditions. Despite this, Colt has a remarkable tolerance to poor drainage and root asphyxia, making it a suitable option for heavy soils with good water availability (Pino *et al*., 2014; Catalán-Paine *et al*., 2025).

Several studies have shown that, under water deficit conditions, cherry trees grafted onto Colt exhibit a significant reduction in vegetative and productive growth, characterized by a smaller leaf area, shorter shoot elongation, smaller fruit size, and yield that is proportional to the severity of the stress (Blaya-Ros *et al*., 2020). In contrast, rootstocks such as Mahaleb and Afgano, considered more drought-tolerant (Pino *et al*., 2014), maintain higher rates of photosynthesis and stomatal conductance under water shortage conditions, which help preserve their physiological activity and yield.

Despite these limitations, the Colt rootstock has been widely adopted in modern fruit growing in Chile. Since 2004, there has been a sustained increase in sales of plants grafted onto Colt, consolidating its position as the most widely planted rootstock in the country in recent years. Between 2008 and 2013, it consistently accounted for more than half of the cherry trees sold annually in nurseries, ranging from 50% to 61% (AGV, 2014). Its use has been concentrated, especially in areas with good water-retaining soils, due to its high compatibility with productive varieties and its resistance to poor drainage and root asphyxia (Ayala, 2009).

Given the scenarios of climate instability and limited water availability, water-use efficiency has become a priority in agriculture. Biomass production is linked to the amount of water available in the soil, and it is estimated that between 50% and 80% of accessible water is used for agricultural activities (Medrano *et al*., 2007). In this context, the use of biostimulants and beneficial microorganisms is a promising strategy for enhancing crop resilience.

Biostimulants derived from seaweed, particularly brown algae such as *Macrocystis pyrifera*, have been shown to improve water-use efficiency, photosynthetic performance, and antioxidant defense in plants. Their effectiveness depends on the extraction method, with mixed hydrolysis (alkaline + enzymatic) being the most effective (Thevanthan *et al*., 2005). On the other hand, plant growth-promoting bacteria (PGPB), such as *Pseudomonas koreensis*, contribute to water-stress tolerance by producing phytohormones, osmolytes, and enzymes, and by improving moisture retention and nutrient absorption (Sati *et al*., 2021).

Although the synergistic application of seaweed extracts and PGPB has shown positive results in herbaceous crops, its effects on woody perennial fruit species such as cherry rootstocks remain unexplored. Thevanthan *et al*. (2005) suggest that this combination can activate complex signaling networks that improve plant resilience to water deficit. Recent studies in tropical palms confirm this potential, showing that combined biostimulants enhance gas exchange, osmotic adjustment, and antioxidant activity under drought (Rodrigues *et al*., 2025). The use of these strategies is particularly relevant in Chile, especially for rootstocks such as Colt, which are widely used in traditional orchards and characterized by their low drought tolerance. In this context, the present study aimed to evaluate the effect of applying extracts of *M. pyrifera*, obtained by alkaline and mixed (alkaline + enzymatic) hydrolysis, in combination with the PGPB bacterium *P. koreensis*, on young Colt rootstock plants. The evaluation was conducted under conditions of abundant irrigation and water deficit, assessing physiological and morphological responses across varying levels of water availability.

2 Materials and methods

2.1 Description of the study site

The study was conducted during the 2025 season, specifically between February and April, in a 65% Rashel mesh shading system located in the nursery of the University of Concepción, Faculty of Agronomy, in Chillán (36°35′46″S; 72°04′49″W), Ñuble Region, Chile. This area has a temperate Mediterranean climate, characterized by hot, dry summers with maximum temperatures exceeding 30°C and winters with temperatures between 5°C and 15°C. Annual rainfall ranges from 800 to 1000 mm, with most falling from May to September (Agrometeorología INIA, 2025).

Two experiments were conducted, using Colt rootstock (*Prunus avium* × *Prunus pseudocerasus*) with an initial growth of 20 cm. The plants were transplanted in January into 2 L pots, using a sandy loam substrate composed of 65% sand, 17.5% clay, and 17.5% silt. This substrate had an organic matter content of 26.5% and was enriched with organic fertilizers containing nitrogen (N), phosphorus (P), and potassium (K) to promote the establishment and initial development of the rootstocks under controlled-shade conditions.

Irrigation was carried out using a micro-sprinkler system, with two inverted medium-range rotary emitters (Aquamic AQ-210, AUTOMAT) per test. This system was installed at approximately 2 meters above ground level, with a 2-meter spacing between emitters, enabling uniform aerial coverage of more than 85% of the substrate surface. Each sprinkler operated at a minimum pressure of 21 psi and a precipitation rate of 5.7 mm h^{-1}, ensuring efficient water distribution during the evaluation period.

2.2 Experimental Design

Two independent trials were conducted, each in an area of 8 m^2 (4 m long × 2 m wide), with irrigation management differentiated between them. The WET trial was conducted under commercial irrigation conditions, maintaining the pots at container capacity, corresponding to a volumetric water content of 0.33 m^3 m^{-3}, which translates to a stem water potential (Ψ_{stem}) of approximately –0.5 MPa. In contrast, the DRY trial also began at container capacity. Still, from the sixth week onward, irrigation was applied only when the pots reached 50% capacity, simulating water-deficit conditions under moderate to severe stress (Ψ_{stem} < –1.1 MPa).

Both trials were set up using a completely randomized block design with four replicates per treatment. Each experimental unit consisted of three Colt rootstock plants, grown in individual pots under controlled conditions. Six experimental treatments were established to evaluate the individual and combined effects of seaweed extracts and PGPB on plant physiological responses under both water conditions.

The Control treatment consisted of plants that received no extracts or bacterial inoculation. Treatment B corresponded to inoculation with strain AG-97 (*P. koreensis*), from the strain collection of the Laboratory of Agricultural Microbiology of the Faculty of Agronomy, University of Concepción. This strain was previously selected from a group of bacterial strains due to its positive effect on vegetative growth and water stress tolerance in a *Prunus* sp. rootstock (López-Fernández,2024). Treatments A1 and A2 corresponded to the application of extracts of *M. pyrifera* obtained by mixed hydrolysis (alkaline + enzymatic) (this process is patented by SICIT group) and by alkaline hydrolysis (this process is patented by Patagonia Biotecnología SPA), respectively. These extracts differ in their chemical characteristics (Table 1). Finally, combined treatments A1B and A2B included the application of the respective extracts (mixed and alkaline hydrolysis) and the inoculation with AG-97 (*P. koreensis*). Bacterial inoculation was performed after transplant (Table 2) by applying 10 mL of bacterial suspension at a concentration of 10^6 colony-forming units (CFU) mL^{-1}.

Seaweed extracts were applied at 0.2% (1.2 mL of extract diluted in 600 mL of water per plant), ensuring uniform coverage of the substrate and adequate availability in the root zone. Applications were made weekly for four consecutive weeks, beginning two weeks after transplanting (Table 2).

2.3 Compatibility between Macrocystis pyrifera extracts and Pseudomonas koreensis

Before establishing the trials and determining the treatments, a compatibility analysis between *M. pyrifera* extracts and the PGPB strain *P. koreensis* AG-97 was conducted under laboratory conditions. To achieve this, the AG-97 strain was multiplied in peptone standard nutrient broth for 36 hours at 25°C in an orbital shaker (60 rpm). Once the medium was colonized, the bacterial suspension was standardized to an optical density of 0.2%. Subsequently, 20 µL aliquots of the colonized culture medium were inoculated onto previously sterilized standard nutrient agar plates, mixed with seaweed extract obtained by mixed extraction (alkaline + enzymatic hydrolysis), and onto another plate with standard nutrient agar mixed with seaweed extract obtained by alkaline extraction. Four replicates (plates) were included for each extract type. The plates were incubated in a growth chamber at 25°C, and bacterial colony growth was assessed.

2.4 Environmental Conditions and Microclimate

Daily data on global solar radiation (W m^{-2}), precipitation (mm), relative humidity (%), and air temperature (°C) were obtained from the "Aeródromo Gral. Bernardo O'Higgins, Chillán" weather station, which belongs to the INIA Agrometeorological Network (Chacón *et al*., 2019; https://agrometeorologia.cl accessed on April 14, 2025), located 3.7 km from the study area. To estimate the effective radiation available for photosynthesis in the experimental area, a portable ceptometer (LP-80, Decagon Devices) was used to measure the Photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}).

Ten PPFD measurements were taken inside the shade house, approximately 0.5 m above the soil surface, near the plant canopy, at the center, edge, and between rows of each trial. Likewise, 10 PPFD readings were taken outside the shade house, under direct radiation conditions, to calculate the percentage of PPFD intercepted in each trial, determined using the following equation:

$$PPFD\ Internal = \left(1 - \frac{PPFD_{in}}{PPFD_{out}}\right) \cdot 100$$

Where PPDF Internal corresponds to the percentage of photosynthetically active radiation (PPFD) intercepted by the canopy inside the shade house; $PPFD_{in}$ represents the average value of readings taken under shade conditions, at the height of the plant canopy; and $PPFD_{out}$ corresponds to the average value of readings taken in full sun conditions (outside the shade house).

At the beginning and end of each physiological measurement, air temperature and relative humidity were recorded inside the shade house, above the plant canopy in each trial. These variables were measured using integrated sensors from a portable porometer (LI-COR LI-600, LI-COR Instruments, Nebraska, USA). The data obtained were used to calculate the air vapor pressure deficit (VPD_{air}) using the equation proposed by Howell and Dusek (1996).

$$VPD_{air} = 0.6108 \cdot exp\left(\frac{17.27 \cdot T}{T + 237.3}\right) \cdot 1 - \left(\frac{RH}{100}\right)$$

Where RH is the relative humidity of the air and T is the air temperature.

2.5 Plants and soil water

Stem water potential was measured in both trials (WET and DRY) from the sixth week of evaluation, at midday (12:00 to 15:00), using a pressure chamber (Model 615, PMS Instruments, Corvallis, USA), following the protocol described by McCutchan and Shackel (1992). Measurements were taken simultaneously in both trials to monitor the physiological response of plants to water deficit in the DRY trial and to abundant commercial irrigation in the WET trial. The first measurement was taken before the irrigation regime was adjusted in the DRY trial, and subsequent measurements were taken every two weeks. In each experimental unit, a leaf was selected and covered with an opaque, airtight bag at least 40 minutes before measurement to equilibrate water potential between the leaf and the stem.

The volumetric content of the soil was monitored only within the effective root zone of the plants in the second block of both trials. Measurements began in the third week of the trials (end of February) and continued until their completion (beginning of April).

Capacitance sensors (model GS1, Decagon Devices, Pullman, WA, USA) installed in the pots at a depth of approximately 10 cm were used for this purpose. The effective root-zone and sensor installation depths were determined based on the previously estimated root development. The data were recorded at 15-minute intervals and stored in a data logger (model Em5b, Decagon Devices, Pullman, WA, USA).

2.6 Plant Physiology and Growth

Stomatal conductance (mmol $m^{-2} s^{-1}$) was determined using a portable porometer (LI-600, LI-COR Biosciences, Nebraska, USA) on three mature leaves located in the upper third of each plant's canopy. Leaf temperature (°C) was recorded using the infrared temperature sensor integrated in the same porometer (LI-600) on a single mature leaf from the same stratum.

The efficiency of photosystem II (PSII) was evaluated using the *Fv*/*Fm* parameter with a chlorophyll fluorescence meter (Pocket PEA, Hansatech Instruments, Norfolk, UK). One mature leaf per plant was selected from the upper third of the canopy and adapted to darkness for 30 minutes using opaque clips (Reyes-Díaz *et al*., 2009). Subsequently, minimum (Fo) and maximum (*Fm*) fluorescence values were recorded, and variable fluorescence (*Fv*) was calculated as Fm – Fo. The photochemical efficiency of PSII was then obtained using the relationship *Fv*/*Fm*.

$$\frac{Fv}{Fm} = \frac{Fm - Fo}{Fm}$$

All physiological measurements were taken simultaneously at midday on mature, healthy leaves without visible damage and were repeated weekly.

During the WET and DRY trials, five measurements of stem growth and leaf fluctuations were taken in each experimental unit. Stem growth was measured from the base of the crown using a manual tape measure to ensure accurate quantification of elongation. Leaf fluctuations, meanwhile, were recorded by counting and direct observation, accounting for changes in leaf number.

2.7 Aboveground and root biomass

To quantify aboveground and root biomass, all plants from both trials were harvested. This process was carried out with extreme care to avoid damage to the plant structures. In the laboratory, the plants were separated into three structures: roots, stems, and leaves, ensuring a correct division between the root system and the stem at the crown level.

Next, the fresh weight of the above-ground structures (stem and leaves) was recorded using a precision balance (±0.001 g). The samples were immediately stored in airtight bags and refrigerated to prevent moisture loss before dry-weight analysis.

To determine the dry weight (constant weight), the samples were dried in a forced-air oven (Heraeus model D-6450, Hanau, Germany) at 65°C. Previously, a representative sample was selected for periodic measurements to establish the time required to reach constant weight. Based on this test, the dry weight stabilized after 3 days of exposure. Consequently, all samples were weighed at the end of this period, and their final dry weight was recorded.

Before weighing, root samples were sieved through a No. 16 laboratory sieve (Standard Testing Sieve, USA) to remove adhering substrate residue that could interfere with accurate dry-weight measurement.

2.8 Water use efficiency and water productivity

At the end of the physiological evaluations, a composite sample was collected from the leaves of the experimental unit for each treatment in both trials to estimate intrinsic water-use efficiency, measured as the difference in the carbon isotope ratio ($\delta^{13}C$). The sampled leaves were mature and fully developed. Once the leaf samples were collected, they were dried in an oven at 65 °C until the sample weight stabilized. After this, the dried samples were ground and sieved to obtain a fine, homogeneous powder. $\delta^{13}C$ and total carbon percentage (%C) were determined using an EA-GSL gas preparation module (Sercon, UK) coupled to a mass isotope ratio spectrometer (20–22 IRMS, Sercon, UK). Before each analysis, an ultra-high-grade reference gas (Ultra High-Grade CO2, Linde Group) was injected to correct for CO_2 drift. A calibrated laboratory standard (Corn Flour SCC2256, Sercon, UK) was analyzed every ten analytical samples. One standard sample was checked every ten analytical samples as an internal control of analytical quality. The stable carbon isotope composition ($\delta^{13}C$) of each sample was determined using the following equation (Farquhar *et al.*, 1989):

$$\boldsymbol{\delta 13C}\ (‰) = \left[\frac{\left(\frac{\mathbf{13C}}{\mathbf{12C}}\right)_{sample} - \left(\frac{\mathbf{13C}}{\mathbf{12C}}\right)_{standard}}{\left(\frac{\mathbf{13C}}{\mathbf{12C}}\right)_{standard}}\right] \cdot \mathbf{1000}$$

Where $13C/12C_{sample}$ and $13C/12C_{standard}$ are the measured 13C/12C ratios for the leaf sample and the PDB standard (Pee Dee Belemnite), respectively.

2.9 Quantification of Cultivable *Pseudomonas* spp.

Root samples were collected at the end of each trial, with all utensils cleaned with 70% ethanol. The samples were then stored at 5°C until transfer to the laboratory, where the total colony-forming units (CFU) of *Pseudomonas* spp. were quantified using a modified method of McSpadden *et al*. (2001).

For microbiological analysis, 1 g of root surface tissue was collected, macerated in a sterile mortar, and transferred to a test tube containing sterile saline (0.89% NaCl). This suspension was kept under constant agitation (150 rpm) at 25 ± 1 °C for 24 hours.

Subsequently, six serial dilutions were prepared in 1.5 mL plastic tubes, each containing 900 µL of sterile saline, and three 10 µL microdroplets of each dilution were seeded onto Petri dishes containing King B (KB) medium. The plates were incubated at 24°C for 24 hours, and the CFU mL^{-1} count was performed, considering only those colonies that showed fluorescence under ultraviolet light (TFX-20-M transilluminator; Vilber Lourma).

2.10 Statistical Analysis

In both trials, the data were subjected to analysis of variance (ANOVA), after verifying the normality of the distribution (Shapiro-Wilks test), homogeneity of error variances (Levene test), and additivity (Tukey test). Differences between means were determined using the LSD test (alpha = 0.05). The relationships among physiological variables, morphological changes, and moisture content were analyzed using linear and quadratic regression. All statistical analyses were performed using RStudio software version 2025.05.0 (RStudio, Posit, USA).

3 Results

3.1 Environmental Conditions and Characterization of Irrigation

During the experimental period (February–April), the microclimatic conditions inside the shade house showed two contrasting phases (Figure 1). Vapor pressure deficit (VPD; Figure 1A) indicated an initial period of high atmospheric demand during early to mid-February, with values exceeding 3.0 kPa, followed by a progressive decline toward the end of the experiment, when VPD decreased to values below 1.5 kPa. The PPFD inside the shade house (Figure 1B) reached its highest values during the first phase of the experiment, exceeding 400 μmol m^{-2} s^{-1}, and remained relatively stable over time. An abrupt decrease in internal PPFD was recorded on March 25, associated with a nearby fire event. Throughout the experiment, internal PPFD represented approximately 20–30% of external radiation, with minor temporal fluctuations. Internal air temperature and relative humidity (Figure 1C) reflected these microclimatic changes. The early phase of the experiment was characterized by higher temperatures (28–35 °C) and lower relative humidity, whereas the later phase, from late March to early April, showed lower temperatures and increased relative humidity, stabilizing around 35–40%.

Under WET conditions, substrate volumetric water content remained close to container capacity throughout the evaluation period (Figure 2A). Moisture values fluctuated between 0.33 m^3 m^{-3} and the total porosity of the substrate (0.56 m^3 m^{-3}), with no sustained periods below container capacity. Temporal oscillations reflected irrigation events, and no consistent differences among treatments were observed, indicating homogeneous water dynamics under abundant irrigation. In contrast, under DRY conditions, soil water dynamics showed three distinct phases (Figure 2B). During the first four weeks, volumetric water content followed a pattern similar to that of the WET trial, remaining between container capacity and total porosity. After the onset of deficit irrigation, substrate moisture declined and fluctuated between container capacity and values close to the permanent wilting point (0.17 m^3 m^{-3}). During this period, irrigation was applied when moisture approached approximately 50% of container capacity, resulting in several events in which the permanent wilting point was reached. Toward the end of the experiment, volumetric water content partially recovered, approaching levels comparable to those observed under WET conditions. Within each irrigation regime, soil water dynamics were similar among treatments.

3.2 Physiological Responses

3.2.1 Stem water potential

In the WET trial (Figure 3A), all treatments showed similar stem water potential (Ψ_{stem}) values at the beginning of the experiment, coinciding with the first application of seaweed extracts on February 18. Throughout the evaluation period, Ψ_{stem} remained within a narrow range, and no statistically significant differences among treatments were detected. At the dates when Ψstem reached its lowest values (March 18 and April 1), the control consistently exhibited more negative values compared to the treatments receiving *M. pyrifera* extracts and/or bacterial inoculation. This pattern persisted in the final evaluation, with biostimulant-treated plants maintaining less negative Ψ_{stem} values than the control.

In the DRY trial (Figure 3B), all treatments displayed a similar water status at the time of the first biostimulant application, with Ψ_{stem} values ranging from –0.55 to –0.65 MPa. Following the onset of deficit irrigation on March 18, Ψstem decreased across treatments. At this date, plants inoculated with *P. koreensis* AG-97 maintained higher

Ψ_{stem} values (approximately –0.55 MPa) compared to the control, which reached values close to –0.70 MPa. At the final evaluation, most treatments exhibited further decreases in Ψ_{stem} as substrate moisture approached 0.25 m^3 m^{-3}. In contrast, treatment A1 maintained less negative values (approximately –0.60 MPa), differing from both A2 and the control. The control treatment reached the most negative Ψ_{stem} values, close to –1.0 MPa and was significantly different from the remaining treatments.

3.2.2 Stomatal conductance

Under WET conditions, significant differences in stomatal conductance (g_s) were detected following the application of biostimulants (Figure 4A). During the last four evaluations (March 15 to April 1), treatments receiving *M. pyrifera* extracts and/or bacterial inoculation showed significantly higher g_s values than the control. The combined treatment A1B consistently exhibited the highest g_s values during this period. Plants inoculated with *P. koreensis* alone also showed increased g_s, reaching values close to 155 mmol m^{-2} s^{-1}. Treatment A1 showed a tendency toward higher g_s values during the final evaluations. In the DRY trial (Figure 4B), g_s declined in all treatments following the onset of water restriction. The control treatment consistently exhibited lower g_s values compared to the remaining treatments, although statistically significant differences were detected only during the last two evaluations. During this period, treatment A1 showed the highest g_s values, exceeding 70 mmol m^{-2} s^{-1}.

A significant relationship between Ψ_{stem} and stomatal conductance was observed in plants treated with the mixed-hydrolysis *M. pyrifera* extract (A1) and its combination with *P. koreensis* strain AG-97 (A1B) (Figure 5). For both treatments, g_s increased as Ψ_{stem} became less negative. A second-degree polynomial regression fitted to the combined dataset of A1 and A1B treatments was statistically significant ($R^2 = 0.36$, $P < 0.001$), describing the relationship between Ψ_{stem} and g_s across the evaluated range of water status.

3.2.3 Photosystem II efficiency

Photosynthetic efficiency (*Fv*/*Fm*) exhibited contrasting temporal patterns between irrigation regimes. Under WET conditions (Figure 6A), *Fv*/*Fm* values remained relatively stable throughout the experiment, fluctuating between 0.79 and 0.82. Following the application of *M. pyrifera* extracts, treatments A1 and A2 showed significantly higher *Fv*/*Fm* values than the control at five weeks, coinciding with a period of reduced PPFD. At the final evaluation, treatments A1 and Bac. exhibited higher *Fv*/*Fm* values compared to the control ($p < 0.05$). Under DRY conditions (Figure 6B), *Fv*/*Fm* displayed greater temporal variability. Treatments A1 and A1B showed higher *Fv*/*Fm* values than the control during mid-March, with statistically significant differences observed at several evaluation dates. Overall, treatments receiving combined applications (A1, A1B and A2B) tended to maintain *Fv*/*Fm* values comparable to or higher than the other treatments, although the timing of these differences varied. At the final evaluation, treatment A1 recorded significantly higher *Fv*/*Fm* values than the control.

3.2.4 Leaf temperature

Leaf temperature differed among treatments and irrigation regimes over the evaluation period. Under WET conditions (Figure 7A), leaf temperature generally followed the temporal pattern of air temperature, with higher values recorded during periods of elevated atmospheric demand. At several evaluation dates, significant differences among treatments were observed. During periods of high air temperature, the control treatment frequently exhibited higher leaf temperatures than plants receiving *M. pyrifera* extracts and/or bacterial inoculation. At specific dates, treatments A1 and A1B showed leaf temperatures comparable to the bacterial inoculation treatment, whereas A2 and A2B displayed intermediate values. Toward the end of the evaluation period, when the air temperature decreased, differences among treatments were reduced or not significant.

Under DRY conditions (Figure 7B), leaf temperature also varied among treatments and dates. During the early phase of the restriction period, leaf temperature values were generally close to or above air temperature, with the control treatment showing the highest values at several dates. Significant differences among treatments were detected at specific evaluations. Treatment A1 tended to show lower leaf temperatures than the control. As atmospheric demand decreased toward the end of the experiment, leaf temperature declined across all treatments, although some differences among treatments were still observable at certain evaluation dates.

3.3 Morphology and Biomass

3.3.1 Steam growth

In the WET trial, stem growth increased steadily throughout the evaluation period relative to the first measurement (Figure 8A). Significant differences among treatments became evident from mid-experiment onward. Approximately four weeks after the first application of *M. pyrifera* extracts, plants receiving the combined treatment A1B exhibited the greatest stem elongation, reaching increases of nearly 17 cm, whereas the control showed an increase of approximately 8 cm. This pattern was maintained in subsequent evaluations. At the end of the trial, A1B reached an average stem growth of about 23 cm, which was significantly higher than the control (approximately 10 cm). The bacterial inoculation treatment (Bac) showed intermediate values, with stem growth close to 19 cm.

In the DRY trial, stem growth was substantially reduced compared with the WET trial (Figure 8B). No significant differences among treatments were detected throughout the evaluation period. All treatments showed limited stem elongation following the onset of water restriction, indicating a generalized reduction in growth under deficit irrigation conditions.

3.3.2 Biomass and biomass allocation

Under WET conditions, significant differences among treatments were observed in biomass allocation (Table 3). In terms of absolute dry weight, treatment A1 exhibited the lowest leaf biomass compared with the control and A2, with differences of approximately 0.05–0.06 g. In contrast, A1 showed the highest root dry weight, exceeding A2 by approximately 0.08 g. No significant differences among treatments were detected for stem dry weight, which ranged between 0.23 and 0.27 g. Relative biomass allocation also differed among treatments under WET conditions (Table 3). The control and A2 treatments showed the highest proportion of leaf biomass, reaching up to 25% in A2, whereas A1 exhibited the lowest percentage in this component. Root biomass accounted for more than 50% of total dry weight in most treatments, with A1 showing the highest allocation (55.7%) and A2 the lowest (48.4%). Stem biomass represented between 23% and 26% of total dry weight across treatments, with no clear differences among treatments. Under DRY conditions, no significant differences among treatments were detected for either absolute dry weight or relative biomass allocation in leaves, stems, or roots (Table 3).

3.4 Carbon assimilation and Isotopic discrimination

Under WET conditions, significant differences in leaf carbon content were observed among treatments (Figure 9A). Treatments A1 and A1B exhibited the highest carbon percentages, exceeding 42%, whereas treatment A2B showed the lowest values, below 40%. Carbon isotope discrimination ($\delta^{13}C$) also differed among treatments under WET conditions (Figure 9B). The most negative $\delta^{13}C$ values (approximately −27.5‰) were recorded in treatments A1 and A1B, while the control exhibited the least negative values (approximately −26‰). Under DRY conditions, no significant differences among treatments were detected for either leaf carbon content or $\delta^{13}C$ (data not shown). A significant relationship between stomatal conductance and carbon isotope discrimination was observed across treatments (Figure 10). A linear regression analysis showed that $\delta^{13}C$ values became more negative as stomatal conductance increased, according to the fitted model $y = -25.91 - 0.02x$ ($R^2 = 0.54$; $p < 0.01$). Treatments exhibiting higher stomatal conductance tended to cluster toward more negative $\delta^{13}C$ values, whereas the control treatment was associated with lower conductance and less negative $\delta^{13}C$ values.

3.5 Compatibility of Macrocystis Extracts and Pseudomonas spp. populations

High compatibility was observed between *M.pyrifera* extracts and the strain *P. koreensis* AG-97 (Table 4). Both extracts, A1 (combined enzymatic and alkaline hydrolysis) and A2 (alkaline extraction), supported bacterial growth under in vitro conditions.

In planta quantification of cultivable *Pseudomonas* spp. populations in the rhizosphere revealed no significant differences among treatments under either irrigation regime (WET or DRY) (Table 4).

4. Discussion

The seaweed biostimulant A1, derived from a combination of enzymatic and alkaline hydrolysis of *M. pyrifera* (i.e. mixed hydrolysis), showed the greatest consistency in improving the drought tolerance of young 'Colt' rootstock plants, regardless of inoculation with the AG-97 strain of *P. koreensi*s. Improving the drought tolerance of the 'Colt' rootstock is paramount for the sustainability of the Chilean sweet cherry industry, as this genotype is highly sensitive to water deficits (Bouljan and Claverie, 2004) and accounts for almost 40% of commercial sweet cherry orchards (Masman, 2024). The results of the present study confirmed the low tolerance of the 'Colt' rootstock to drought conditions, as Control plants exhibited no reduction in stomatal conductance in response to a substantial decrease in soil water content and plant water status, as it was evidenced by the absence of a significant correlation between stem water potential (Ψ_{stem}) and stomatal conductance (g_s) throughout the experimental period. Under dry conditions, withholding irrigation reduced substrate moisture from the container capacity (ΘCC ~0.35 m^3 m^{-3}) to values near the permanent wilting point (Θ_{PWP} ~0.15 m^3 m^{-3}) over three consecutive one-week periods. A lack of effective stomatal control to reduce transpiration when atmospheric evaporative demand remained high (2.0-3.5 kPa) caused the largest drop in Ψ_{stem} in Control plants among the treatments.

While Control plants experienced a maximum water stress severity of -1.1 MPa, A1 plants exhibited a Ψ_{stem} value of -0.7 MPa, which was very close to the optimum plant water status for *Prunus* spp. for the environmental conditions of early April, according to the Ψ_{stem} baseline developed by McCutchan and Shackel (1992). Furthermore, in the WET trial, where the substrate moisture was continuously maintained at very high values (between container capacity and saturation, θ_{SAT} ~0.47 m^3 m^{-3}), Ψ_{stem} was consistently above -0.7 MPa, confirming that the application of mixed-extracted seaweed biostimulants (A1 and A1B) successfully maintained 'Colt' plants under optimum water conditions. Notably, most treatments exhibited higher plant water status than the Control at the final measurement date, except for the alkaline-extracted seaweed biostimulant (A2). In this study, A2 plants registered the lowest Ψ_{stem} and root dry weight among all treatments. While differences in root dry weight were only detectable under WET conditions, differences in Ψ_{stem} were found under DRY conditions. In the present study, plants subjected to the DRY regime were similarly irrigated to those in the WET trial for 82% of the evaluation period, including the period of highest seasonal root growth rates (spring, October to November). This indicates that A2 plants exhibited reduced root development under well-irrigated conditions, which could have affected water uptake in the DRY plot, where water availability was limited. The absence of substantial treatment effects on root dry weight under DRY conditions does not indicate that biostimulants had no effect on root development and growth. These results suggest that DRY conditions were severe enough to reduce root carbon allocation in all treatments. In fact, root dry weight across all treatments under DRY conditions was 20% less than under WET conditions.

In this context, one of the most important traits for drought tolerance is the ability to close stomata when soil water content is low. The application of both mixed-extracted seaweed biostimulants, A1 and A1B, induced stomatal closure when the severity of plant water stress increased. This was evident by analyzing the significant quadratic relationship between Ψ_{stem} and gs ($P<0.0001$; $R^2=0.36$). As this study was conducted under semi-shading conditions, the relationship between Ψ_{stem} and g_s was not as strong as has been reported for sunlit leaves in other species of *Prunus* spp. (Spinelli *et al.*, 2016). When A1 and A1B-treated plants showed Ψ_{stem} values close to –1.0

MPa, g_s was reduced to almost zero, limiting transpiration and plant dehydration under the DRY conditions. These results may indicate that mixed-extracted seaweed biostimulants were associated with stronger stomatal regulation as water stress intensified, consistent with previously reported drought-mitigation effects (Grammenou et al., 2023). The chemical analyses of seaweed extracts showed that mixed-extracted biostimulants had 73% and 42% higher concentrations of betaines and mannitol, respectively, than the alkaline-extracted biostimulants (A2 and A2B). The elevated mannitol content provides a plausible osmotic mechanism for enhanced stomatal regulation under drought conditions, as mannitol can accumulate in the guard cell apoplast and modulate aperture through osmotic adjustment of guard cells (Patel *et al*., 2021; Grammenou *et al*., 2023). In addition, seaweed extracts promote osmolyte accumulation and osmoprotection; their application increases proline and related osmolytes in several species, contributing to cellular osmotic adjustment during water or salt stress (Chen *et al*., 2021; Islam *et al*., 2020).

Pseudomonas koreensis has been shown to enhance drought tolerance and modulate ABA-related gene expression in Arabidopsis, suggesting a potential role for this PGPB in influencing stomatal ABA pathways. However, the covariance analysis showed that both mixed-extracted seaweed biostimulants exhibited the same relationship between Ψ_{stem} and g_s. This finding may indicate that the AG-97 strain of *Pseudomonas koreensis* was not responsible for inducing the stomatal closure at low Ψ_{stem} in A1-treated plants, but this does not exclude microbial influence on stomatal signaling. In fact, in the present study, the inoculation with AG-97 strain, either alone or combined with a seaweed biostimulant, enhanced g_s under well and deficit-irrigated conditions. Under WET conditions, the BAC and A1B treatments exhibited 2- to 3-fold higher gs than the Control for 70% of the evaluation period. Under DRY conditions, the BAC biostimulant was the only treatment that consistently showed higher g_s than the Control. Regardless of the irrigation strategy, the greater effect of the AG-97 strain on g_s was detected one month after inoculation. In the last two measurements (first week of April), a decrease in g_s was observed in the BAC plants, although these treatments continued to show significantly higher g_s values than the

Control. The strain AG-97 has been identified as a producer of AIA (Reyes-Castillo *et al*., 2019), as reported for other *P. koreensis* strains (Guo *et al*., 2021). High concentrations of AIA have been associated with increased stomatal conductance in plants by stimulating ethylene synthesis, which inhibits ABA-mediated stomatal closure (Tanaka et al., 2006). This provides a plausible explanation for the higher g_s observed under high moisture and the reduced stomatal responsiveness of AG-97 inoculated plants under moderate water deficit. Additionally, the strain's lower stimulatory effect on stomatal opening at the end of the WET and DRY tests may be due to decreased bacterial activity as autumn approaches, coinciding with reduced ambient and, therefore, soil temperatures. In general, mesophilic bacteria such as *P. koreensis* exhibit maximal metabolic activity and growth at temperatures of 30 °C (BacDive, 2026). In April, the maximum temperature fell to 20 °C; therefore, the soil in the trial is estimated to have been near 16 °C, which is below the optimal temperature for this type of PGPB. Consequently, these results indicate that although AG-97 did not alter the regression pattern between Ψ_{stem} and g_s, inoculation stimulated g_s, likely through IAA-ethylene signaling and metabolic enhancement modulated by seasonal soil temperature.

Inoculation of the AG-97 strain of *P. koreensis* in WET plants treated with mixed seaweed extracts (A1B) produced a 200% increase in stem elongation compared to the control treatment. Greater stem elongation is relevant in environments with moderate to low light intensity, as in this study, as it favors light interception. These results demonstrate that strain AG-97 increased plant height in A1-treated plants, as no significant difference in stem length was observed between A1 and Control. Although A1B had a higher stem length, the dry weight of the stem was similar in all treatments, indicating that the increase in elongation was not due to greater carbohydrate accumulation. Under conditions of high-water availability ($\Psi_{stem} > –0.6$ MPa), organ growth depends mainly on turgor, which allows elongation without necessarily increasing dry biomass (Taiz *et al*., 2017; Blum, 2017). It should be noted that no variation in Ψ_{stem} was observed between treatments under humid conditions, suggesting that the increase in stem length was probably not associated with greater water accumulation. Therefore, the greater elongation in A1B plants may be due to more elastic cell walls, since softer walls with equal turgidity may exhibit higher growth rates. Several studies have addressed the impact of seaweed extracts and PGPB on cell wall loosening, suggesting that organic compounds such as polyphenols, polysaccharides, and hormones (e.g., AIA) could stimulate the synthesis of cell wall-softening proteins such as expansins, which would explain the greater stem length in A1B plants (Majda and Robert, 2018).

Despite A1 plants showed no higher stem length than Control, the WET treatment seems to have changed the allocation of carbohydrates compared to A2. Thus, A1 plants accumulated less dry mass in leaves than A2, but more in roots at the end of the experiment. Surprisingly, A1 and A1B exhibited 2% higher percentage of total carbon in leaves than A2. The percentage of carbon (%C) measured by IRMS quantifies the proportion of carbon atoms in a homogenized, dried sample and is not influenced by tissue thickness, leaf mass area, or mineral elements, but only by the relative abundance of carbon-containing molecules. Oven-drying at 60°C for 72 hours removes water but retains all mineral ashes and structural compounds. Consequently, the resulting dry mass reflects not only carbon-rich organic matter but also non-carbon mineral content and structural density. Therefore, A1 leaves were likely to be thinner, less dense, and to contain a lower mineral ash content, which results in a lower oven-dry mass than A2 leaves. This means that A1 leaves probably exhibited a higher proportion of carbon-rich compounds, such as soluble carbohydrates, phenolics, cuticular lipids, or lignin, than A2 leaves. The lower leaf construction costs of A1 plants indicate reductions in carbon and mineral investment in foliage. This suggests a reallocation of resources toward belowground growth, consistent with the observed 7% higher relative root dry biomass in A1 compared with A2. Although not directly measured, such a shift in resource allocation could contribute to enhanced root development and, potentially, greater water uptake capacity.

Although plants treated with A1 and A1B showed greater drought tolerance due to more efficient stomatal closure, surprisingly, these treatments also had the lowest intrinsic water use efficiency, as assessed by ^{13}C isotope discrimination in leaves under WET conditions, but not under DRY conditions. The fact that the Control in the WET trial exhibited greater water use efficiency is closely related to its lower stomatal conductance throughout the experiment. Under conditions of high substrate moisture between field capacity and saturation, it is possible that the plants experienced periods of root hypoxia, so maintaining high stomatal conductance is key to drying the soil and improving aeration. In addition, during the warmest months of the trial (February-March), the leaf temperature of the Control plants was 1–1.5°C higher than that of the treatments with biostimulants, particularly A1B. These results clearly indicate that maintaining high transpiration rates in plants treated with biostimulants, in a very moist substrate and at temperatures near 30°C, is a positive attribute, even if water use efficiency is low. Surprisingly, the lower stomatal conductance of the Control plants was not low enough to reduce carbon assimilation in the leaves. This was reflected in a higher dry matter percentage in the Control leaves, approximately 20% higher than in the A1 plants in the WET trial. Given that the Control plants ended the trial with the fewest leaves, this greater accumulation of dry matter would correspond to the distribution of the same amount of carbohydrates in fewer sinks (leaves). The study was conducted in a shade house covered with 20% shade cloth, which means that the plants grew under an average light intensity 50% lower than the saturation point for *Prunus* spp. (600 µmol m^{-2} s^{-1}) (De Jong, 1983). For this reason, the fact that the leaves of the Control were denser than those of the A1 plants could have generated greater resistance to CO_2 diffusion through the mesophyll, affecting photosynthetic efficiency. In contrast, the less dense leaves of the A1 plants favor their expansion to capture light and dissipate heat. This would explain why the A1 plants exhibited slightly higher *Fv*/*Fm* values than the Control plants at the end of the experiment, both in the WET and DRY tests.

5 Conclusions

The present study shows that the mixed-hydrolysis seaweed biostimulant A1 was the most effective treatment for enhancing drought tolerance in young 'Colt' rootstocks.

This genotype is highly sensitive to water deficits and key to the sustainability of the Chilean sweet cherry industry. The A1 demonstrated consistent effectiveness in enhancing plant water status under drying conditions. This enabled ‘Colt’ plants to maintain optimal stem water potential values for *Prunus* spp., while effectively promoting stomatal closure as soil moisture levels decreased. This improved stomatal regulation was probably linked to the biochemical composition of mixed-extracted seaweed products, particularly their high mannitol content. Although inoculation with *Pseudomonas koreensis* AG 97 did not modify the response curve of stomatal conductance to plant water stress, this strain enhanced stomatal conductance and stimulated stem elongation under high-moisture conditions. However, these PGPB physiological effects were diluted as soil temperatures declined in autumn. Finally, A1-treated plants exhibited functional leaf traits that were advantageous under shadehouse conditions, including lower leaf dry matter content, greater leaf expansion, reduced leaf temperature, and slightly higher photosynthetic efficiency. Because oven-drying retains mineral residues, the lighter A1 leaves indicate lower construction costs, suggesting that resources were redirected below-ground. This interpretation aligns with the 7% higher relative root biomass in A1 plants and may reflect enhanced capacity for root development and water uptake. The results indicate that mixed-extracted seaweed biostimulants, either alone or in combination with PGPR inoculation, are a promising biologically grounded strategy to strengthen the drought resilience and physiological performance of 'Colt' rootstocks under contrasting water regimes.

6 Figures Legends

Figure 1. Internal environmental conditions of a shade house located in Chillán, Ñuble Region. (A) Internal vapor pressure deficit (VPD), (B) Internal photosynthetic photon flux density (PPFD) and its percentage relative to external conditions, and (C) Internal air temperature and relative humidity.

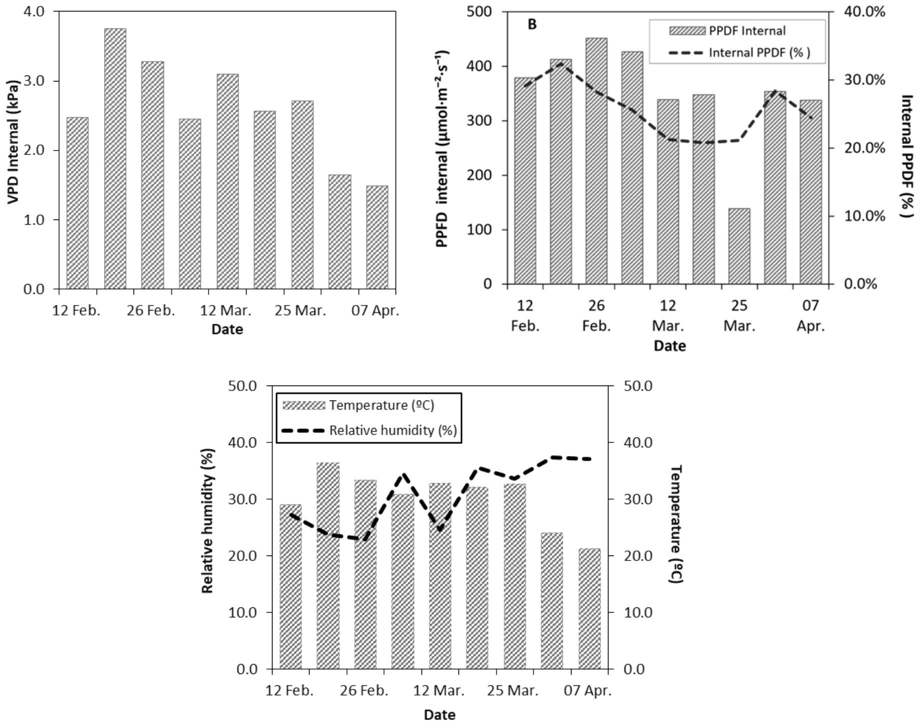

VPD Internal (kPa)
4.0
3.0
2.0
1.0
0.0
12 Feb.
26 Feb.
12 Mar.
25 Mar.
07 Apr.
Date
B
PPDF Internal
Internal PPDF (%)
PPFD internal (µmol·m⁻²·s⁻¹)
Internal PPDF (%)
500
400
300
200
100
0
40.0%
30.0%
20.0%
10.0%
0.0%
12 Feb.
26 Feb.
12 Mar.
25 Mar.
07 Apr.
Date
Temperature (ºC)
Relative humidity (%)
Relative humidity (%)
Temperature (ºC)
50.0
40.0
30.0
20.0
10.0
0.0
12 Feb.
26 Feb.
12 Mar.
25 Mar.
07 Apr.
Date

Figure 2. Volumetric water content in the substrate over time in ‘Colt’ cherry rootstocks under two irrigation regimes: (A) WET (maintained at container capacity) and (B) DRY (irrigated at 50% capacity from the fourth week). The dotted line at 0.33 m³·m⁻³ indicates container capacity, while the lines at 0.17 m³·m⁻³ and 0.56 m³·m⁻³ indicate the permanent wilting point and total porosity, respectively, according to the methodology of Peverill *et al*. (1999)

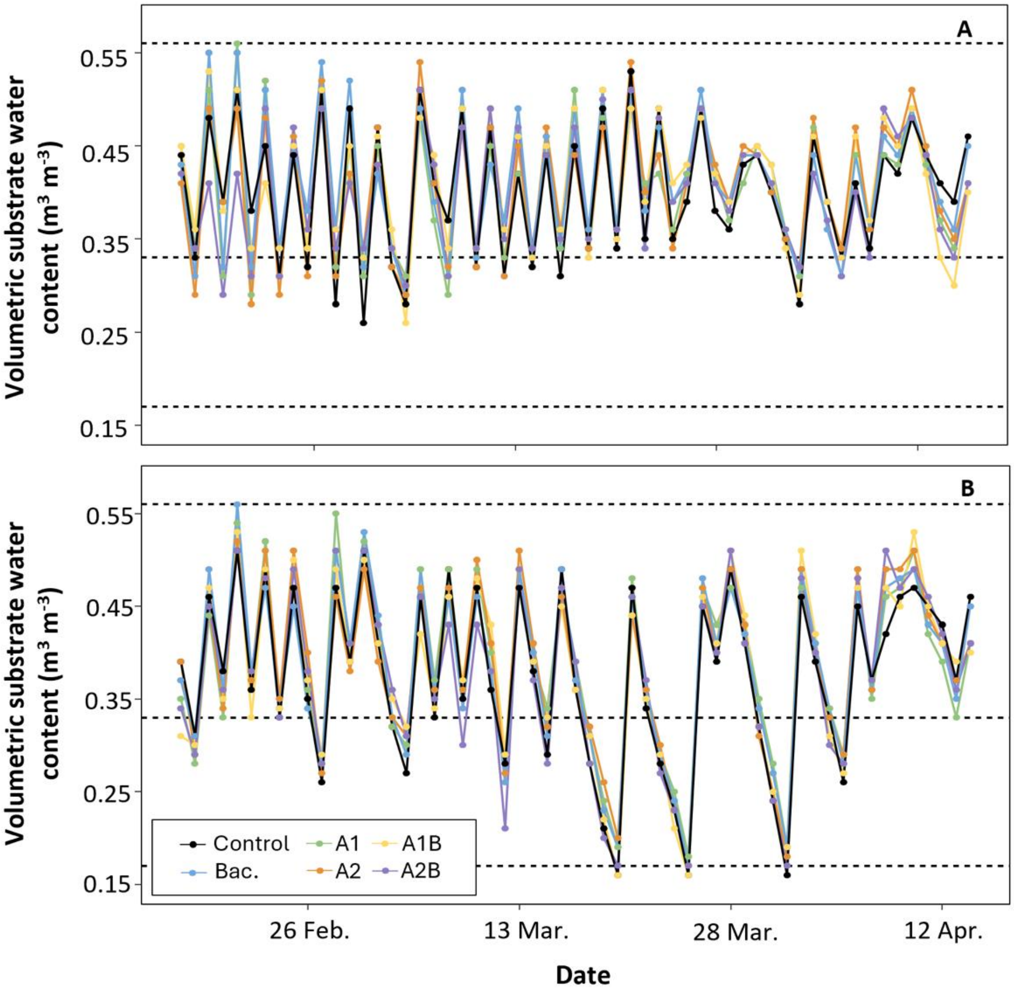


Figure 3. Stem water potential (MPa) measured at midday (12:00–15:00) in cherry rootstock 'Colt' under two irrigation regimes: (A) WET (maintained at container capacity) and (B) DRY (irrigated at 50% container capacity from week four). Treatments included Control (no application), Bac. (*Pseudomonas koreensis* AG-97), A1 and A2 (A1: mixed extraction enzymatic hydrolysis + alkaline; A2: alkaline extraction), and combined treatments A1B and A2B (extracts plus bacterial inoculation). Different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p < 0.05$).

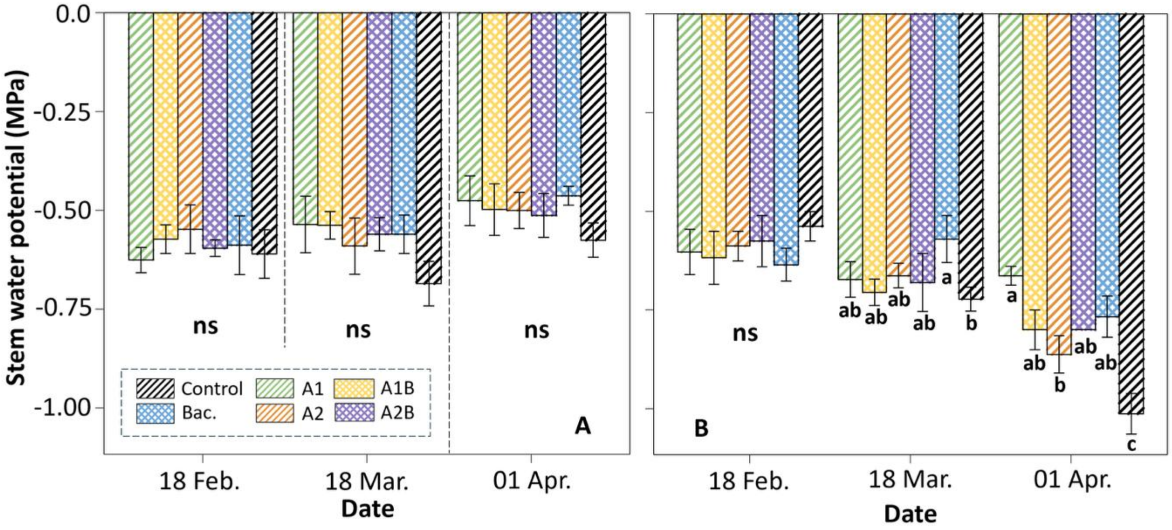


Figure 4. Stomatal conductance (mol·m⁻²·s⁻¹) measured in cherry rootstock 'Colt' under two irrigation regimes: (A) WET (maintained at container capacity) and (B) DRY (irrigated at 50% container capacity from week four). Treatments included Control (no application), Bac. (*Pseudomonas koreensis* AG-97), A1 and A2 (A1: mixed extraction enzymatic hydrolysis + alkaline; A2: alkaline extraction), and combined treatments A1B and A2B (extracts plus bacterial inoculation). Different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p < 0.05$).

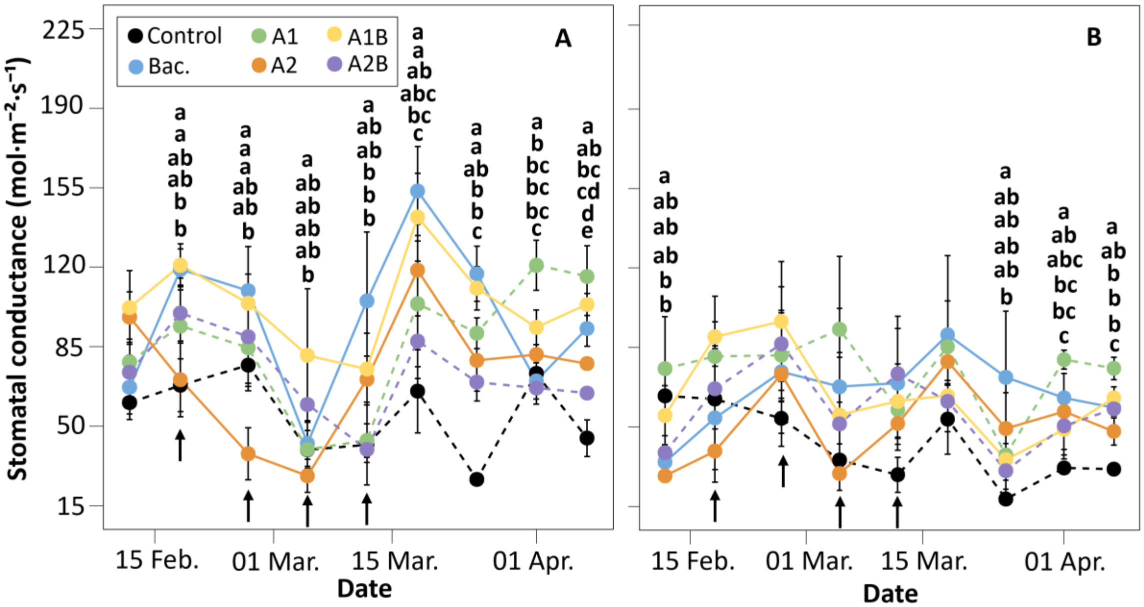


Figure 5. Relationship between stem water potential and stomatal conductance in cherry rootstock Colt under treatments with *Macrocystis pyrifera* extract obtained by mixed hydrolysis + Alkaline (A1) and its combination with *Pseudomonas koreensis* strain AG-97 (A1B). A second-degree polynomial regression was fitted to the data ($R^2 = 0.36$, $P < 0.001$), with the equation $y = –551.9 x^2 – 508.9 x – 4.66$.

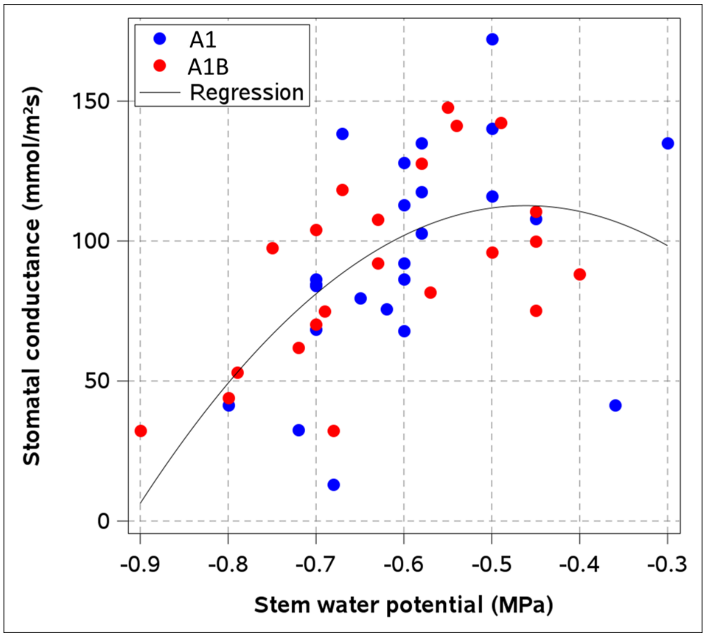


Figure 6. Photosynthetic efficiency (*Fv*/*Fm*) measured in cherry rootstock 'Colt' under two irrigation regimes: (A) WET (maintained at container capacity) and (B) DRY (irrigated at 50% container capacity from week four). Treatments included Control (no application), Bac. (Pseudomonas koreensis AG-97), A1 and A2 (A1: mixed extraction enzymatic hydrolysis + alkaline; A2: alkaline extraction), and combined treatments A1B and A2B (extracts plus bacterial inoculation). Black arrows indicate biostimulant application dates. Different letters indicate statistically significant differences between treatments according to Fisher's LSD test ($p < 0.05$).

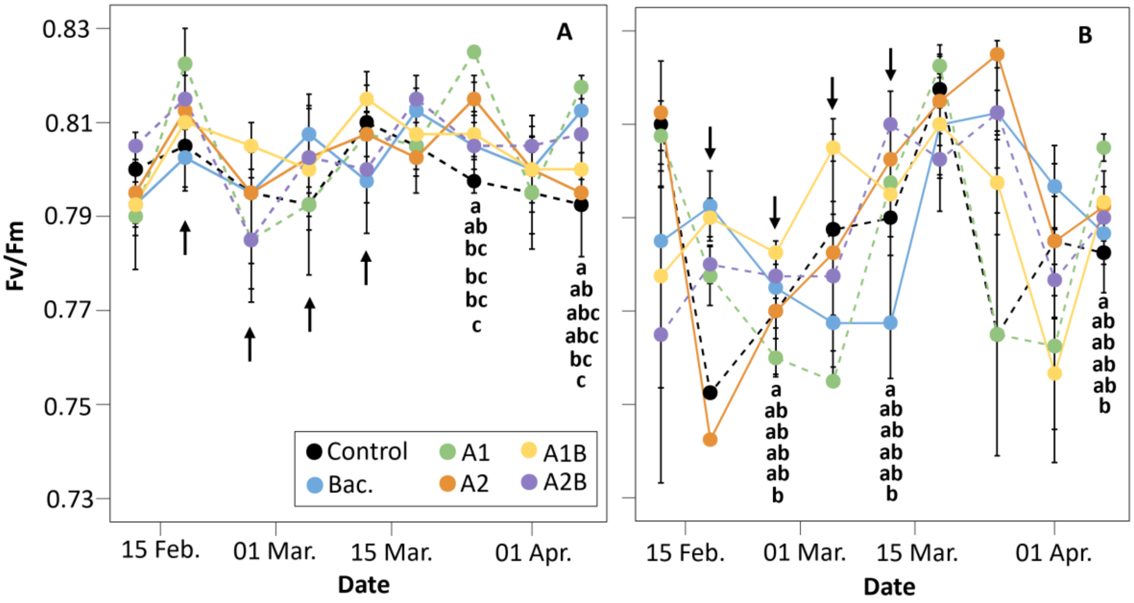


Figure 7. Leaf temperature (°C) of ‘Colt’ plants under two irrigation regimes: (A) WET (maintained at container capacity) and (B) DRY (irrigated at 50% container capacity from week four), measured from February 26 to April 7. Bars represent six treatments: Control (no application), Bac. (*Pseudomonas koreensis* AG-97), A1 and A2 (A1: mixed extraction enzymatic hydrolysis + alkaline; A2: alkaline extraction), and combined treatments A1B and A2B (extracts plus bacterial inoculation). The red dashed line indicates the average air temperature recorded on each date. Error bars represent standard error, and lowercase letters denote statistical differences among treatments ($p < 0.05$); ns indicates non-significant differences.

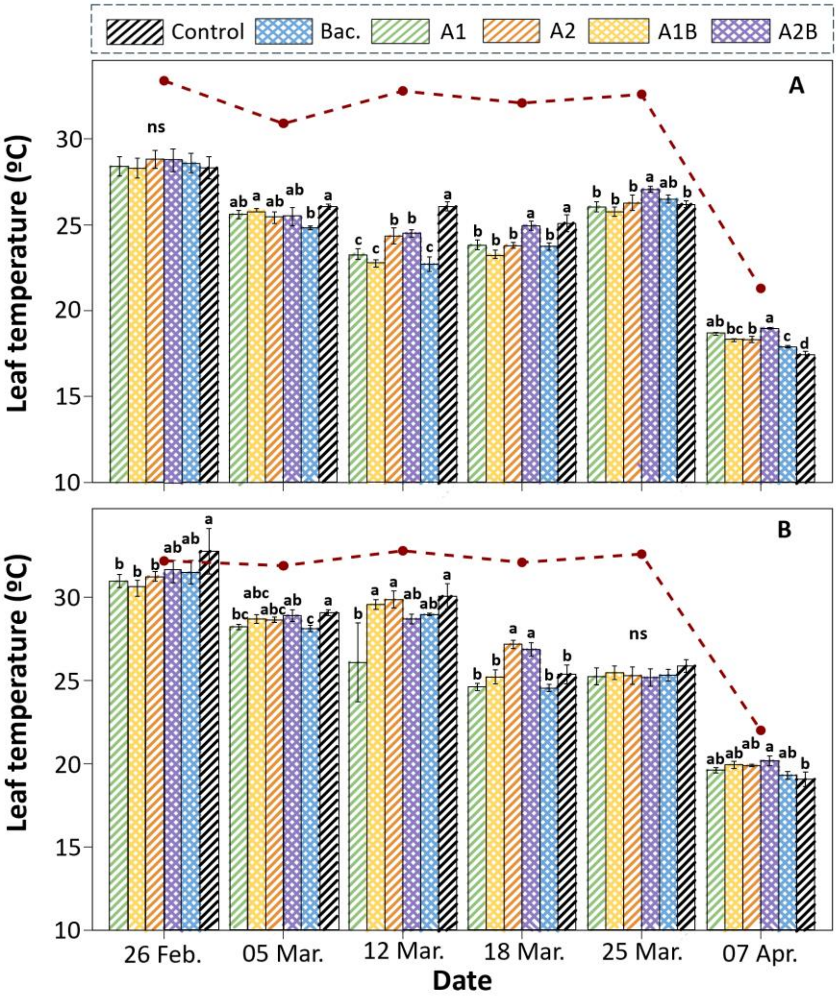


Figure 8. Stem growth (cm) in cherry rootstock 'Colt' under two irrigation regimes: (A) WET (maintained at container capacity, 0.33 m³·m⁻³) and (B) DRY (irrigated at 50% container capacity from week four), expressed relative to the first measurement. Treatments included Control (no application), Bac. (*Pseudomonas koreensis* AG-97), A1 and A2 (A1: mixed extraction enzymatic hydrolysis + alkaline; A2: alkaline extraction), and combined treatments A1B and A2B (extracts plus bacterial inoculation). Black arrows indicate biostimulant application dates. Lowercase letters above bars denote statistical differences among treatments ($p < 0.05$).

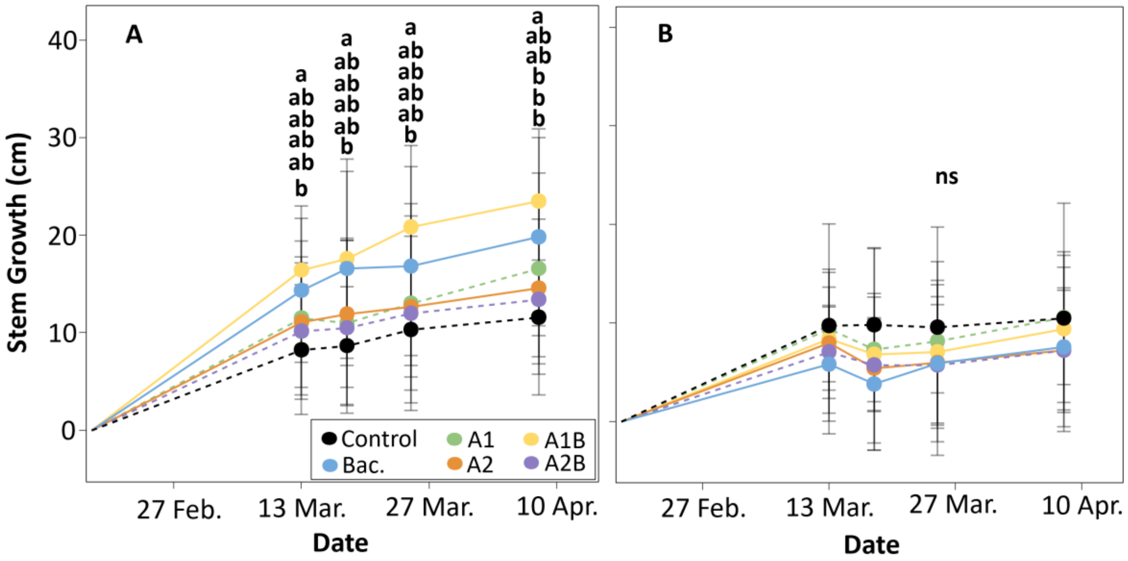


Figure 9. Leaf dynamics (change in number of leaves relative to the previous measurement) in ‘Colt’ plants under two irrigation regimes: (A) WET (maintained at container capacity, 0.33 $m^3 \cdot m^{-3}$) and (B) DRY (irrigated at 50% container capacity from week four), evaluated between March 13 and April 8. Bars represent six treatments: Control (no application), Bac. (*Pseudomonas koreensis* AG-97), A1 and A2 (A1: mixed extraction enzymatic hydrolysis + alkaline; A2: alkaline extraction), and combined treatments A1B and A2B (extracts plus bacterial inoculation). Error bars indicate standard error, and lowercase letters denote statistical differences among treatments according to Fisher’s LSD test ($p < 0.05$); ns indicates non-significant differences.

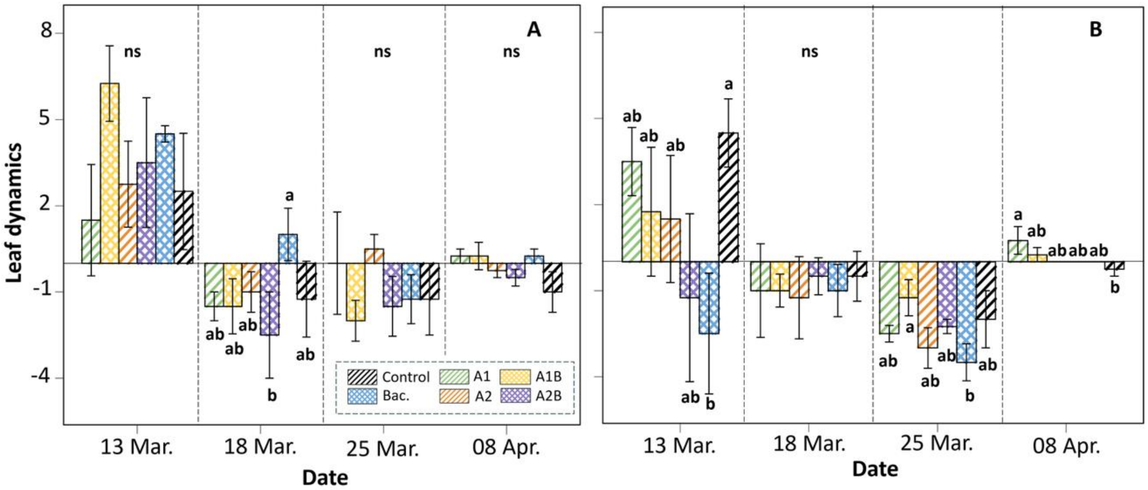


Figure 10. (A) Carbon content (%) and (B o Bac???) ^{13}C discrimination in cherry rootstock 'Colt' under different treatments in the WET trial. Treatments included A1 and A2 (A1: mixed extraction enzymatic hydrolysis, + alkaline; A2: alkaline extraction), Bac. (~~*Pseudomonas*~~ *P. koreensis* AG-97), combined treatments A1B and A2B (extracts plus bacterial inoculation), and Control (no application). Error bars represent standard error, and different lowercase letters indicate significant differences among treatments according to Fisher's LSD test ($p < 0.05$).

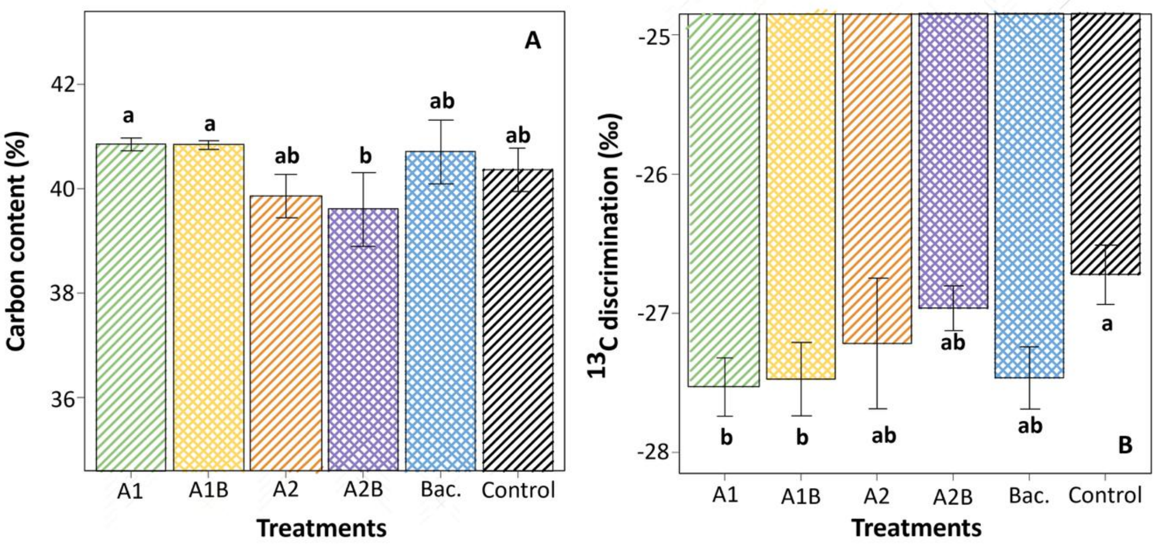
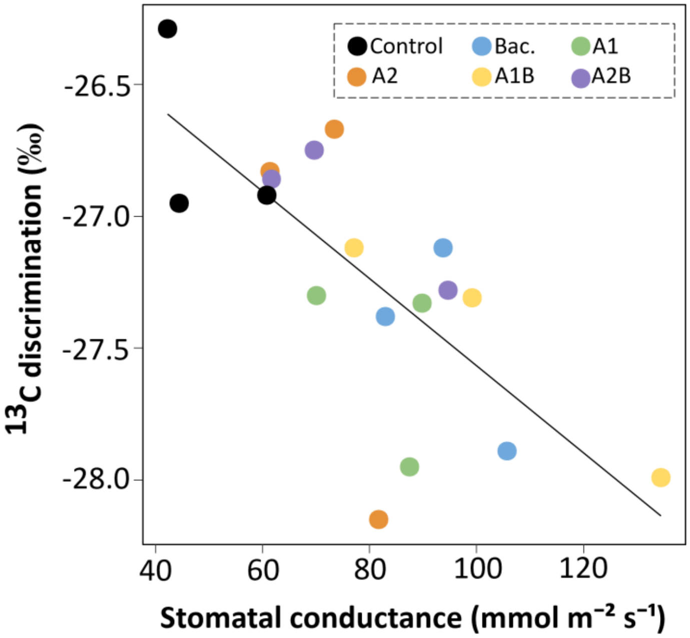


Figure 11. Relationship between stomatal conductance (mmol m^{-2} s^{-1}) and ^{13}C discrimination in cherry rootstock 'Colt' under different treatments. The fitted model is described by the equation $y = -25.91 - 0.02x$ ($R^2 = 0.54$; $p < 0.01$). Treatments included Control (no application), Bac. (*Pseudomonas koreensis* AG-97), A1 and A2 (A1: mixed extraction enzymatic hydrolysis, + alkaline; A2: alkaline extraction), and combined treatments A1B and A2B (extracts plus bacterial inoculation).



7 Tables

Table 1. Chemical composition of *Macrocystis pyrifera* extracts obtained by mixed hydrolysis (A1: alkaline + enzymatic) and alkaline hydrolysis (A2).

Chemical Composition of *Macrocystis Extracts*		
	A1 (Alkaline + Enzymatic)	A2 (Alkaline)
pH	7 - 7.5	10.5 - 12.5
Alginate Acid (mg kg^{-1})	15,000	2,500
Betaines (mg kg^{-1})	4.5	2.6
Total Carbon (%w w^{-1})	1.5	0.4
Total Nitrogen (%w w^{-1})	0.1	4.0
Mannitol (mg kg^{-1})	1000	700

*Treatments that included bacterial inoculation were Bac. (*Pseudomonas koreensis* AG-97), A1B, and A2B (A1B and A2B: seaweed extracts plus bacterial inoculation). Treatments that included seaweed extract applications were A1, A2, A1B, and A2B (A1 and A2: *Macrocystis pyrifera* extracts via mixed and alkaline hydrolysis). The timing of inoculation and extract applications was identical across treatments.

Table 2. Application Timeline for Bacterial Inoculation and Seaweed Extract Applications.

Type of application	Application number	Date
Bacterial inoculation	1	Feb. 7th
Macrocystis pyrifera extract applications	1	Feb. 18th
Macrocystis pyrifera extract applications	2	Feb. 26th
Macrocystis pyrifera extract applications	3	Mar. 5th
Macrocystis pyrifera extract applications	4	Mar. 12th

Table 3. Absolute and relative dry weights of leaves, stems, and roots in cherry rootstock 'Colt' under two irrigation regimes: WET (maintained at container capacity, 0.33 $m^3 m^{-3}$) and DRY (irrigated at 50% container capacity from week four). Treatments included Control (no application), Bac. (*Pseudomonas koreensis* AG-97), A1 and A2 (A1: mixed extraction enzymatic hydrolysis, + alkaline; A2: alkaline extraction), and combined treatments A1B and A2B (extracts plus bacterial inoculation). Error bars represent standard error, and different lowercase letters indicate significant differences among treatments according to Fisher's LSD test ($p < 0.05$).

	Absolute dry weight (g)			Relative dry weight (%)		
	Leaves	Stem	Roots	Leaves	Stem	Roots
WET						
Control	0.24 a	0.26	0.50 ab	23.9% a	26.0%	50.1% ab
Bac.	0.23 ab	0.23	0.54 ab	23.0% ab	23.3%	53.8% ab
A1	0.19 b	0.24	0.56 a	19.9% b	24.5%	55.7% a
A2	0.25 a	0.27	0.48 b	25.0% a	26.6%	48.4% b
A1B	0.21 ab	0.24	0.54 ab	21.6% ab	24.0%	54.4% ab
A2B	0.23 ab	0.24	0.53 ab	23.3% ab	23.9%	52.8% ab
DRY						
Control	0.21	0.36	0.43	20.7%	36.9%	43.4%
Bac.	0.16	0.38	0.46	16.3%	37.8%	46.0%
A1	0.22	0.39	0.39	22.1%	38.5%	39.4%

A2	0.22	0.28	0.5	21.8%	28.1%	50.1%
A1B	0.15	0.43	0.43	14.9%	42.5%	42.6%
A2B	0.15	0.47	0.38	14.8%	47.3%	38.0%

Table 4. Microbial count of *Pseudomonas* spp. obtained from the in vitro evaluation with *Macrocystis pyrifera extracts*. Result expressed in Colony Forming Units (Log10 CFU mL^{-1}).

Treatment	Bacterial count (Log$_{10}$ CFU mL^{-1})
Pseudomonas koreensis AG-97	8.50
Pseudomonas koreensis AG-97 and A1	8.11
Pseudomonas koreensis AG-97 and A2	8.32

*A1 corresponds to extract of *Macrocystis pyrifera* obtained via mixed hydrolysis + alkaline; A2 corresponds to the extract of *Macrocystis pyrifera* obtained via alkaline.

8 Conflict of Interest

Macarena Cruzat-Hermosilla and Marcelo Bintrup are employees of Patagonia Biotecnología S.p.A. The mixed hydrolysis (alkaline + enzymatic) process used in treatment A1 is covered by the international patent PCT/IB2025/055451, held by SICIT Group. The alkaline hydrolysis process used in treatment A2 is covered by the Chilean patent No. 54.947, held by Patagonia Biotecnología S.p.A. The remaining authors declare no other commercial or financial relationships that could be construed as a potential conflict of interest.

9 Author Contributions

MCH: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing; TA: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing; CAO: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing; MG: Methodology, Formal analysis, Writing – review & editing; MB: Methodology, Writing – review & editing; MGa: Methodology, Writing – review & editing.

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