Uniciencia Vol. 39(1), pp. 1-12, January-December, 2025
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DOI: https://dx.doi.org/10.15359/ru.39-1.12
E-ISSN: 2215-3470

Comparison between two propagation methods in ‘Reina Claudia’ plum (*Prunus domestica* L.)

Comparación entre dos métodos de propagación en el ciruelo ‘Reina Claudia’ (Prunus domestica L.)

Comparação entre os métodos de propagação na ameixa ‘Reina Claudia’ (Prunus domestica L.)

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Received: Sep/15/2024 • Accepted: Apr/22/2025 • Published: Nov/30/2025

Abstract

[Objective] The propagation method of plum trees is crucial for production success, as it likely influences plant compatibility and performance. The objective of this research was to evaluate two propagation systems of the ‘Reina Claudia’ plum tree variety, comparing bud grafting and cuttings, to identify the method most compatible with the variety. **[Methodology]** Since 2018, an experiment has been conducted in Sesquilé, Cundinamarca, to compare the two propagation methods of ‘Reina Claudia’ plum trees (*Prunus domestica* L.). The experiment followed a completely randomized design. One method involved bud grafting onto a ‘common white’ peach rootstock (*Prunus persica* L.), while the other used cuttings from the same plum variety. **[Results]** Significant differences were observed in the main branch diameter, with trees propagated by cuttings (TPC) reaching 31.62 mm, compared to 17.74 mm in those propagated by grafting (TPG). Water potential in TPC trees ranged from 0 to -107.25 kPa, while in TPG trees, it ranged from 0 to -58.25 kPa. Stomatal conductance values were higher in TPG (0 to 879 mmol m^{-2} s^{-1}) than in TPC (149.0 to 761.31 mmol m^{-2} s^{-1}). The propagation method influenced both water potential and chlorophyll content in the plum trees. **[Conclusions]** The TPC trees exhibited greater vegetative growth, including increased height, leaf area, crown diameter, and crown volume. However, TPG trees demonstrated greater precocity.

Keywords: chlorophyll; soil water potential; stem diameter; stem water potential; stomatal conductance.

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DOI: https://dx.doi.org/10.15359/ru.39-1.12
E-ISSN: 2215-3470

Resumen

[Objetivo] El método de propagación del cultivo de ciruelo es importante, pues de este probablemente dependerá el éxito de la producción. Debido a esto, el objetivo de esta investigación fue evaluar dos sistemas de propagación de ciruelo, variedad 'Reina Claudia', mediante injerto de yema y a través de estacas, con el fin de obtener plantas compatibles con la variedad. **[Metodología]** Por lo anterior, desde el año 2018 se lleva a cabo un experimento en el municipio de Sesquilé, Cundinamarca, con el propósito de comparar, mediante un diseño completamente aleatorizado, dos métodos de propagación del ciruelo 'Reina Claudia' (*Prunus domestica* L). Uno, con injerto de yema, en un patrón de durazno blanco común (*Prunus persica* L.), y otro, por medio de estacas de la misma variedad del ciruelo. **[Resultados]** Hubo diferencias significativas entre el diámetro de la rama principal de los árboles propagados por estaca (APE) (31.62 mm), y los árboles propagados por injerto (API) (17.74 mm). Los APE tuvieron un potencial hídrico que osciló entre 0 y -107.25 kPa, mientras que en los API varió entre 0 y -58.25 kPa. La conductancia estomática alcanzó mayores valores en los API (0 a 879 mmol m^{-2} s^{-1}), que en los APE (149.0 y 761.31 mmol m^{-2} s^{-1}). El método de propagación afecta el potencial hídrico y el contenido de clorofila de los árboles de ciruelo. **[Conclusiones]** Los APE mostraron un mayor crecimiento vegetativo, altura del árbol, área foliar, diámetro y volumen de la copa, no obstante, los API mostraron una mayor precocidad.

Palabras clave: clorofila; conductancia estomática; diámetro de tallo; potencial hídrico del tallo; potencial hídrico del suelo.

Resumo

[Objetivo] O método de propagação da ameixeira é importante, pois dele provavelmente dependerá o sucesso da produção, portanto o objetivo desta pesquisa foi avaliar dois sistemas de propagação da ameixeira, variedade 'Reina Claudia', por enxertia de gema e através de estacas, a fim de obter plantas compatíveis com a variedade. **[Metodologia]** Portanto, desde 2018, é realizado um experimento no município de Sesquilé, Cundinamarca, com o objetivo de comparar dois métodos de propagação da ameixeira 'Rainha Claudia' (*Prunus domestica* L) através de um delineamento inteiramente casualizado. Uma por enxertia de gemas, sobre porta-enxerto de pêssego branco comum (*Prunus persica* L.), e outra por estaquia da mesma variedade de ameixa. **[Resultados]** Houve diferenças significativas entre o diâmetro do galho principal das árvores propagadas por estaquia (TPC) (31.62 mm) e das árvores propagadas por enxertia (TPG) (17.74 mm). O TPC apresentou potencial hídrico que variou entre 0 e -107.25 kPa, enquanto, no TPG, variou entre 0 e -58.25 kPa. A condutância estomática atingiu valores maiores no TPG (0 a 879 mmol m^{-2} s^{-1}) do que no PSA (149.0 e 761.31 mmol m^{-2} s^{-1}). O método de propagação afeta o potencial hídrico e o teor de clorofila das ameixeiras. **[Conclusões]** O TPC apresentou maior crescimento vegetativo, altura das árvores, área foliar, diâmetro da copa, volume da copa, porém o TPG apresentou maior precocidade.

Palavras-chave: clorofila; condutância estomática; diâmetro do caule; potencial hídrico do caule; potencial hídrico do solo.

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Introduction

Globally, China leads plum production with over 6 million tons, followed by countries such as Romania, Serbia, the United States, Iran, Turkey, India, Chile, Morocco, and Ukraine (Afanador-Barajas *et al*., 2022). Similarly, the European plum is widely cultivated in Colombia, where annual production reaches 18,733 tons across approximately 1,402 hectares. The department of Boyacá stands out as the main producer, contributing 70% of the national output, with a yield of 13.3 t ha^{-1} in 2022 (Agronet, 2024). However, despite being a producing country, Colombia ranks 44th in global production and has seen a rise in plum imports during 2019 and 2020, which poses a threat to local production and indicates that the domestic market does not meet the growing demand for consumption (Serrano *et al*., 2021).

Plum trees can be divided into two main groups of varieties: European (*Prunus domestica*) and Asian (*Prunus salicina*). However, there is another species of plum, Prunus americana, which belongs to the North American genetic pool, as suggested by Topp *et al*. (2012). Asian varieties are more popular due to their lower chilling requirements and earlier maturity (Gutiérrez-Villamil *et al*., 2024; Bauchrowitz *et al*., 2022). The 'Reina Claudia' variety (*Prunus domestica* L.) produces yellow fruits with fine, juicy pulp and requires deep, loamy soils with good organic matter content and a low water table due to its high sensitivity to root hypoxia (Guerra & Casquero, 2008).

Success in orchard production and fruit quality largely depends on selecting suitable plant material and implementing effective agronomic management, which ensures good soil anchorage, optimal root development, efficient water and nutrient absorption, hormone synthesis, and adequate carbohydrate reserve accumulation, resulting in vigorous trees and high-quality production (Devin *et al*., 2023). In this context, the choice of propagation method is crucial for achieving successful fruit crops (Javid *et al*., 2022).

Propagation by grafting involves combining different genotypes by joining plant material from two different plants (Feng *et al*., 2024). The basal part, the rootstock, supports and develops the root system. It is the more resilient section, serving as nutritional and growth support for the graft (Loupit *et al*., 2023). The upper part, known as the scion, forms the crown, comprising stems, branches, leaves, flowers, and fruits (Mudge *et al*., 2009). The tissues that fuse in grafting are the vascular cambium and callus, which differentiate into new tissues and organs. Compatibility is essential for successful fusion, ensuring the plant does not experience morphological or physiological changes (Javid *et al*., 2022).

In Colombia, the most commonly used rootstock for grafting peach and plum varieties is the 'common white' peach (*Prunus persica* L.), which is resistant to nematodes and adapts well to altitudes between 1,800 and 3,000 m. It also has low chilling requirements (200 to 400 hours) (Coronado *et al*., 2015). Rootstocks are selected based on traits absent in the scion variety, such as drought tolerance, disease resistance, and the ability to develop a healthy root system (Chen *et al*., 2024).

Asexual propagation involves taking a stem, root, or leaf segment from the parent plant and placing it in favorable conditions to promote the development of adventitious roots and the growth of the aerial part, ultimately forming a new plant (Javid *et al*.,

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E-ISSN: 2215-3470

2022). The descendant plant may differ in morphology and production from the parent due to phenotypic variations induced by environmental factors (Jin *et al*., 2022). This propagation method offers advantages, including the preservation of genotypes without genetic variability and ease of propagation, compared to grafting. However, it has drawbacks, including a low root production rate, which results in a fragile root system and the potential for disease transmission from the donor plant to the new plant (Javid *et al*., 2022).

Despite the widespread use of these methods, limited knowledge exists regarding the effects of the interaction between grafting and cutting propagation on the development of plum trees. Therefore, two propagation systems for the 'Reina Claudia' plum variety were evaluated.

Methodology

- *Location*

The experiment was conducted since 2018 in Sesquilé, Cundinamarca, Colombia, on an area of 0.132 ha, located at coordinates 5.04518° N and 73.79120° W. The climate is cold and dry, with an average temperature of 12.7 ºC, as recorded by a portable meteorological station, WS-GP1 (AT delta-T Devices, USA), located on the farm. The average potential evapotranspiration (ETo) was calculated using the Penman-Monteith equation (Wang *et al*., 2021), and was 2.63 mm day^{-1} for 2023.

- *Materials Used*

The zone contained twelve rows of eight Reina Claudia plum trees (*Prunus domestica* L.), planted in clay-loam soil. Before planting on August 16, 2017, branches were selected from 70-year-old 'Reina Claudia' plum trees before bud sprouting to distinguish between vegetative and flowering branches. The latter were identified by the brown edges of their scales. Buds were collected, hydrated, wrapped in damp newspaper and black plastic bags, and stored at 4°C for three months. On December 20, 2017, they were grafted using the budding method onto one-year-old 'common white' peach rootstocks. Simultaneously, 100 cuttings from the same refrigerated branches were planted in plastic bags containing a substrate of sand, rice husks, and humus. After the cuttings sprouted, developed true leaves, and established sufficient roots, they were transplanted to the field (cutting-propagated trees), alongside the grafted trees, in a three-row planting arrangement with a 4 × 4 m spacing (722 trees ha^{-1}).

- *Experimental Design*

The experimental design was a randomized complete block design, structured according to the slope of the terrain and the distribution of trees on the plot. Two treatments were evaluated, each with four replications, forming eight experimental units (EUs), each containing 11 to 13 trees. The first treatment involved trees propagated by grafting (TPG), and the second involved trees propagated by cuttings (TPC). A drip irrigation system was used, with two 8 L h^{-1} emitters per tree. All EUs were irrigated at 100% of the ETc throughout the crop cycle. The volume of water applied was controlled by adjusting the irrigation time, with a frequency of every two days.

- *Response Variables*

The trunk and main branch diameters of the cuttings and grafts were measured every 30 days from the time of planting in 2018 to the present. Eleven and thirteen

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DOI: https://dx.doi.org/10.15359/ru.39-1.12
E-ISSN: 2215-3470

trees were selected and marked per replicate (44 and 52, respectively, per treatment). Measurements were taken using a precision caliper with a 0.01 mm resolution (Mitutoyo Corporation, Japan). The shaded area under the trees at solar noon on clear days was measured using the dot grid method (Wünsche *et al*., 1995), which accounts for varying shade percentages. Two trees per replicate (eight per treatment, 16 in total) were assessed. Additionally, tree height and crown diameter were measured annually.

Chlorophyll content (CC) was determined in four leaves per replicate (16 per treatment) using a SPAD-502 PLUS chlorophyll meter (Konica Minolta Inc., Japan) at solar noon, simultaneously with the measurement of stomatal conductance (g_s) and stem water potential (Ψstem) in mature leaves. Stomatal conductance (g_s) was assessed in four leaves per replicate (16 per treatment) using an SC-1 porometer (Decagon Devices Inc., Pullman, WA, USA). Ψstem was determined using the Scholander Model 600 pressure chamber (PMS Instrument Company, Albany, OR, USA), following the methodology of Scholander *et al*. (1965), on two leaves per replicate located in the lower third, on the north side of eight trees per treatment, every 8 days. Saturated leaf osmotic potential (Ψo) was measured as the leaf solute concentration in 10 leaves per replicate using a Wescor Vapro 560 vapor pressure osmometer (Wescor Inc., Logan, UT, USA). Leaf dry mass content and hydration status were assessed following the methodology of Rodríguez *et al*. (2012).

Soil water potential (Ψs) was measured every three days using eight Watermark 200SS granular matrix sensors (Irrometer Company Inc., CA, USA), with two sensors per replicate installed at depths of 20 cm and 40 cm, positioned 25 cm from the emitter and the drip line. Sensor data was collected with a Watermark handheld reader (Irrometer Company Inc., CA, USA). Volumetric soil moisture content (θv) was determined three times in 2023, using eight disturbed soil samples, one per experimental plot, taken from the vicinity of the irrigation bulb at a depth of 0-25 cm around a representative tree per treatment.

- *Data Analysis*

Statistical analysis was conducted using SAS OnDemand for Academics (SAS Institute Inc., Cary, NC). The Student's t-test for independent samples ($\alpha = 0.05$) was used to compare means.

Analysis and Results

The 2023 study focused on analyzing the water status and vigor of plants in two plum propagation systems. Irrigation doses were applied according to crop requirements, and due to high rainfall, no water deficit occurred throughout the year (Figure 1A). In 2023, the volumetric soil moisture content (θv) in the grafted trees (TPG) ranged from 32.5% to 45.7%, significantly exceeding the field capacity (26.9%). In contrast, the moisture levels in the cuttings (TPC) ranged between 30.5% and 47.7%. However, these values are approximate due to the coefficient of variation (CV) of the averages, which ranged between 5.8% and 9.2% for the TPG and 4.5% to 15.5% for the TPC, indicating relatively low precision.

Soil water potential (Ψs) in the TPG remained between 0 and -58.25 kPa throughout the year, while in the TPC, it ranged between 0 and -107.25 kPa, with recovery during rainfall events (Figure 1A).

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This variation in water potential was attributed to fluctuations in soil moisture. In the TPG, higher moisture content was observed, likely due to runoff, as the trees in this treatment were located in a lower part of the plot. Additionally, the moisture sensors did not detect variations in soil moisture content during drying and wetting cycles, likely because the clay-loam soil has a high moisture retention capacity. This resulted in a minimal reduction in moisture content during dry periods, which escaped detection by the installed Watermark sensors due to their slow response times and low sensitivity in the wet range (Ψs > -10 kPa) (Thompson *et al*., 2006).

Regarding plant water status, no significant differences were observed between the two propagation systems in most measurements over time, including midday stem water potential (Ψstem), saturated osmotic potential (Ψo), and chlorophyll content (CC). Osmotic potential values for TPC were 194.2±66.3 mmol kg^{-1}, while for TPG they were 209.1±47.9 mmol kg^{-1}. However, differences in Ψstem and CC were noted at specific times, attributed to variations in soil moisture and the earlier development of TPG trees. The dry mass content of the leaves was 0.37 and 0.31 g for TPG and TPC, respectively, while the leaf moisture averaged 65.4 and 71.1% for TPG and TPC, respectively. These findings suggest that the two propagation methods generally respond similarly, with some exceptions during the tree growth cycle. Notably, significant differences were observed in Ψstem on days 58, 74, 130, 173, and 236 after planting (Figure 1B), and in CC on days 58, 74, 81, 172, 177, 236, 242, 244, 247, 251, 261, 292, and 356 (Figure 2).

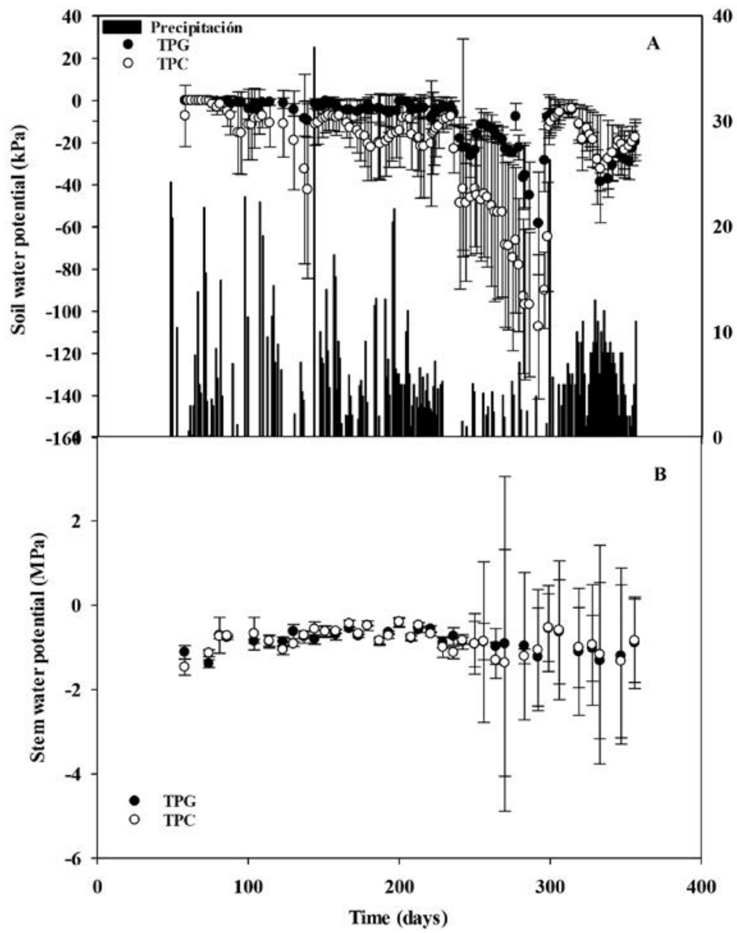

Figure 1. *A) Soil water potential (Ys) and B) stem water potential (Ystem) in a crop plum tree propagated by graft (TPG) and tree propagated by cutting (TPC). Values correspond to the averages of two watermark sensors per replicate, eight per treatment, and to the averages of two leaves in each replicate, eight per treatment, from February 27 (day 58) to December 22, 2023 (day 356), respectively, for each of the potentials.*
Note: derived from research.

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E-ISSN: 2215-3470

These differences were mainly attributed to two factors. First, Ψstem differences resulted from soil moisture variations, which may have been lower in TPC trees, as they were located in the upper part of the land, and potentially received less runoff compared to the lower-lying TPG trees. Second, throughout the experiment, a consistent trend of greater precocity was observed in TPG trees, evidenced by earlier natural leaf wilting as they entered dormancy. This phenomenon was first observed in TPG trees, resulting in a chlorotic appearance and a decrease in chlorophyll content. However, when flower buds sprouted in TPG, new vegetative shoots appeared, reversing the situation.

It is likely that in TPG trees, hormone production and distribution, such as cytokinins and auxins, are affected by the union of different tissues (rootstock and scion). Cytokinins, produced in the roots, promote cell growth and division, especially in buds. If grafting promotes higher cytokinin production or transport to the aerial parts of the tree, it may induce earlier bud sprouting (Monden *et al*., 2022). Additionally, the greater precocity observed in TPG may be related to increased ethylene sensitivity or production, which accelerates leaf abscission and dormancy onset, potentially due to the graft's influence on the tree's hormonal balance (Khan *et al*., 2024). Abscisic acid (ABA) is a hormone closely linked to bud dormancy and stress adaptation. In TPG trees, increased levels of ABA may lead to an earlier onset of dormancy, characterized by chlorosis and leaf abscission. However, once dormancy is achieved, the subsequent decline in ABA levels, along with the activation of cytokinins, could promote the early sprouting of both floral and vegetative buds (Yamane *et al*., 2023).

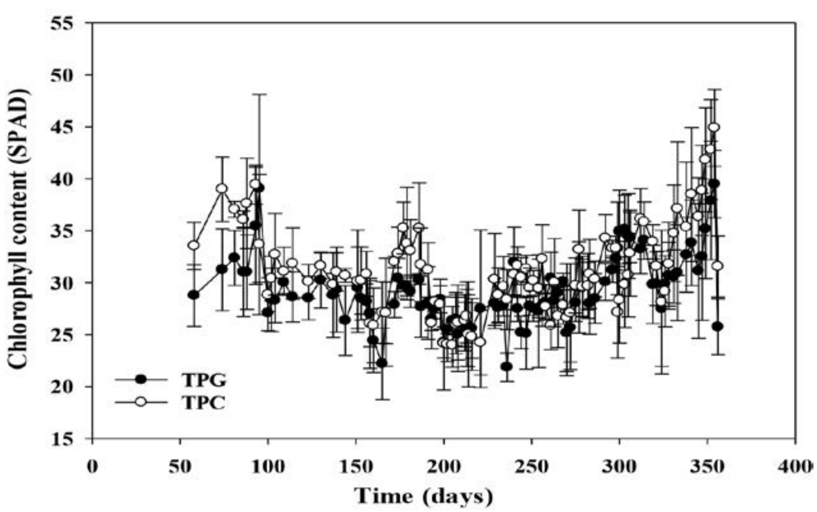

Figure 2. *Chlorophyll content of plum trees propagated by grafting (TPG) and cuttings (TPC) in 2023, since February 27. The values correspond to the averages of two leaves in each repetition, eight per treatment.*
Note: derived from research.

Stomatal conductance (g_s) and leaf moisture percentage, based on fresh weight, were higher in TPC, with significant differences between treatments. g_s was notably higher in TPC at 208, 222, 229, 236, 242, 250, 256, and 264 days after planting, attributed to the larger leaf area, which facilitated greater stomatal opening, transpiration, and moisture retention (Pathare *et al*., 2020). Midday g_s values ranged between 149.0 and 761.31 mmol m^{-2} s^{-1} in TPC and 149.0 and 879.7 mmol m^{-2} s^{-1} in TPG, respectively. Throughout the measurement period, TPG generally showed

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E-ISSN: 2215-3470

lower g_s values than TPC, with differences increasing over time as plants responded to water retention. The larger shaded area in TPC (0.57±0.25 m^2) resulted in higher g_s and transpiration, whereas TPG, with a considerably smaller leaf area (0.12±0.04 m^2), exhibited stomatal regulation, leading to reduced photosynthesis. In this case, photoassimilates were preferentially allocated to primary metabolite synthesis at the expense of secondary metabolites and transpiration, preventing turgor loss (Nadal-Sala *et al*., 2021; Mellisho et al., 2012).

When analyzing the correlation between temperature and stomatal conductance, the relationship was significant at a 99% confidence level for 126 degrees of freedom, with a critical r-value at 1% of 0.22824. For TPG, r = 0.7192, and TPC, r = 0.8012 (Figure 3).

Over five years, the vegetative development and vigor of the plants showed that the trunk diameter of TPC was 2.55 times greater (45.45 mm) than that of the 'common white' peach rootstock (*Prunus persica*), which measured 17.8 mm. Significant differences have been observed since planting on October 1, 2018 (day zero), with the disparity progressively increasing over time as the trees matured, reaching its peak on December 9, 2023, at day 1,896 (Figure 4A). The height and crown diameter of TPG were 0.77±0.04 and 0.42±0.02 m, respectively, while those of TPC were 1.72±0.12 and 1.05±0.08 m, respectively. These differences may be due to the fact that cuttings, taken directly from the mother plant, retain genetic traits that promote more vigorous growth. In contrast, rootstocks are often selected for their ability to withstand specific environmental conditions or regulate plant size, which can limit their growth. This discrepancy in diameter growth is also attributed to differences in the structure of vascular tissues and hormone signaling between the graft and rootstock (Rasool *et al*., 2020).

Similarly, the diameter of the main branch in TPC was 1.78 times larger (31.62 mm) than that of the TPG (17.74 mm), with significant differences ($P \leq 0.05$) evident from the first year of planting, which progressively increased over time (Figure 4B). This

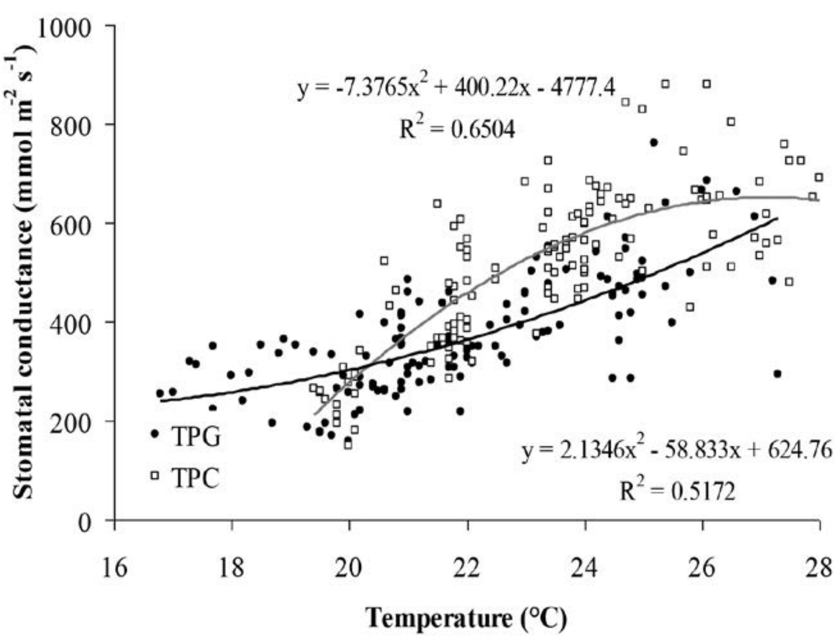

Figure 3. *Correlation between stomatal conductance and temperature of plum trees propagated by grafting and cutting in 2023. Values correspond to the means of 4 leaves in each replicate, 16 per treatment.*
Note: derived from research.

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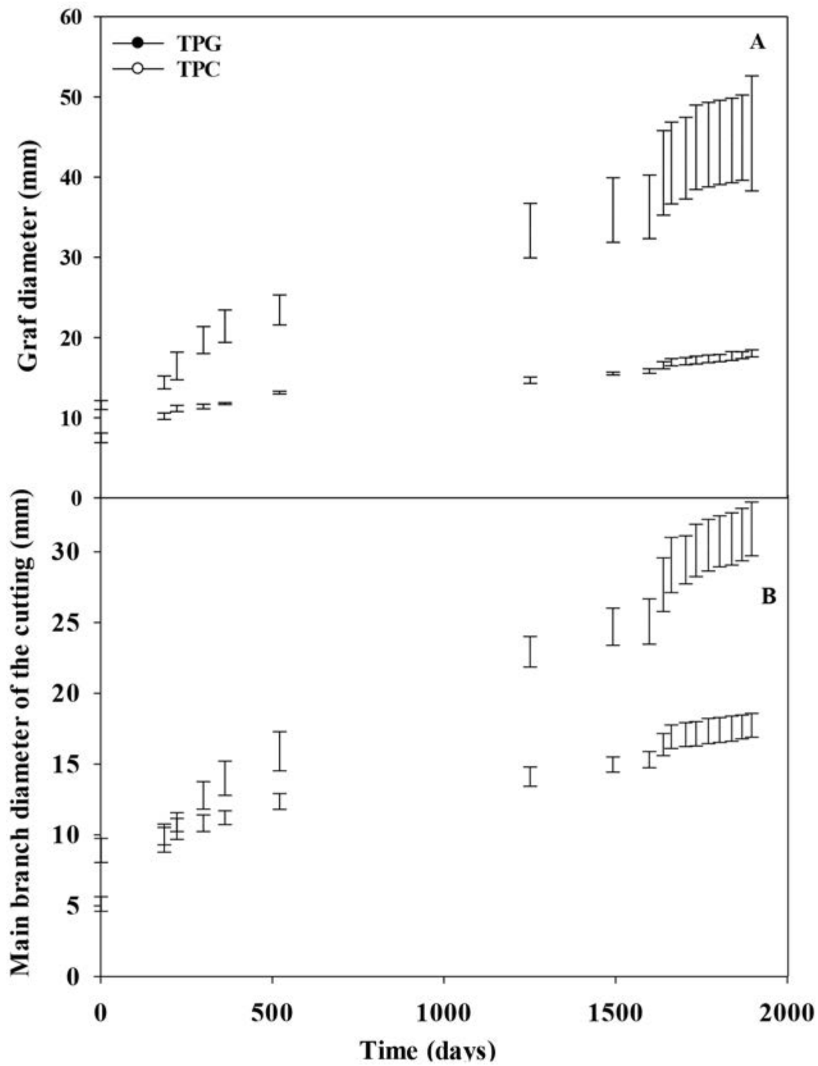

Figure 4. *Evolution of A) graft diameter and B) diameter of the main branch of the cutting in trees propagated by grafting (TPG) and trees propagated by cutting (TPC). Day zero corresponds to October 1, 2018, the date of planting of the trees in the field, and day 1896 corresponds to December 9, 2023.*
Note: derived from research.

restricted nutrient flow and reduced growth rates (Li *et al*., 2022).

The study also examined flowering and fruit set, finding that the TPG began flowering earlier than the TPC. However, despite the TPC having a higher flowering rate, its fruit set percentage was lower, potentially due to the self-sterility of the 'Reina Claudia' plum (data not shown).

difference may result from variations in the growth vigor between the two propagation methods. TPC is known to develop more extensive root systems, facilitating greater water uptake and promoting increased trunk and branch diameter. In contrast, TPG may experience physiological incompatibilities between the rootstock and graft, leading to

Conclusions

Trees propagated by cuttings exhibited larger trunk diameters, while trees propagated by grafting demonstrated earlier flowering. Water potential, chlorophyll content, and stomatal conductance in plum trees were influenced by the propagation method.

At present, there is no definitive preference between the two propagation systems in terms of productivity. Further trials are recommended to assess long-term productivity and fruit quality.

Conflict of Interest

-The authors declare no competing interests.

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Author contribution statement

All the authors declare that the final version of this paper was read and approved.

Authors and CRediT Roles: J.V.S.: Conceptualization, Methodology, Writing - Original Draft, Supervision. J.A.H.: Data Curation, Formal Analysis, Writing - Review & Editing, Visualization. L.B.P.: Validation, Investigation, Software, Resources, Writing - Original Draft.

The total contribution percentage for this paper was as follows: J.V.S. 40 %., J.A.H. 30 % and L.B.P. 30 %.

Data availability statement

The data supporting the results of this study will be made available by the corresponding author, **[J.A.H.]**, upon reasonable request.

Preprint

A preprint version of this article was deposited at: https://doi.org/10.5281/zenodo.13765884

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DOI: https://dx.doi.org/10.15359/ru.39-1.12
E-ISSN: 2215-3470
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