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Abstract

Water scarcity increasingly threatens viticulture, but the drought-response strategy of patrimonial cultivars such as País remains poorly characterized. This study evaluated intra-cultivar variation in drought response among País plants from Arica, Huasco, and Limarí, using Cabernet Franc as a reference, under semi-controlled pot conditions during a five-week dry-down followed by rewatering. Relative pot water content, stem water potential, and stomatal conductance were monitored repeatedly, and provenance-specific stomatal thresholds (P12, P50, and P88) were estimated from nonlinear vulnerability curves. Water deficit caused a progressive decline in pot water content, stem water potential, and stomatal conductance in all materials. Cabernet Franc maintained the highest maximum stomatal conductance, whereas País showed a more conservative stomatal pattern. Provenance differences within País were clearest at P50: Huasco reached 50% stomatal reduction at more negative stem water potentials than Arica and Limarí, while P12 and P88 largely overlapped. After rewatering, stem water potential recovered more rapidly than stomatal conductance, with Arica and Limarí showing the strongest hydraulic recovery. These results demonstrate physiologically relevant intra-cultivar variation in País and indicate that drought-response strategy in this cultivar is not explained by source-site aridity alone, but by provenance-specific regulation of stomatal decline and recovery.

Keywords: *Vitis vinifera*; País; intra-cultivar variability; drought stress; stomatal conductance; stem water potential; hydraulic thresholds; recovery after rewatering

1. Introduction

Water scarcity is one of the main constraints on grapevine cultivation, and this limitation is expected to intensify under current climate change scenarios. In *Vitis vinifera*, seasonal water requirements for wine production commonly range from 300 to 700 mm [1], often exceeding the

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natural water availability of agroecological regions where viticulture is economically important, particularly in Mediterranean-type climates. This situation is especially relevant in Chile, where the high evaporative demand of the main viticultural areas makes irrigation necessary in most vineyards. One of the few exceptions is the cultivar Listán Prieto (syn. País in Chile, Criolla Chica in Argentina, Negra Criolla in Peru, and internationally recognized as Mission or Criolla), a traditional grapevine that has been cultivated for nearly five centuries under rainfed conditions, particularly in the Maule and Biobío valleys. This cultivar is included within the special denomination of origin "Secano Interior," together with Cinsault (Decreto N°56, MINAGRI), and has recently experienced renewed interest due to the cultural and patrimonial value of its wines [2].

Despite this historical adaptation to rainfed systems, cv. País remains particularly vulnerable to extreme climatic events, such as the megadrought that has affected central Chile during the last decade [3]. In this context, reduced precipitation, altered rainfall patterns, and increasing temperatures negatively affect vine productivity, fruit quality, and, in severe cases, plant survival. Consequently, adaptive strategies are urgently needed to sustain viticultural systems under increasing water limitation. Although the expansion of irrigated areas may appear to be an obvious solution, it is not always feasible from an economic, social, or environmental perspective. Therefore, the use of plant material with enhanced drought resistance, either through cultivars or rootstocks, has emerged as a highly relevant adaptive strategy [4]. However, the adoption of new cultivars is often constrained by economic and cultural factors, as well as by regulatory restrictions associated with denominations of origin. Under these circumstances, the exploration of intra-cultivar variability offers a promising alternative that may improve drought adaptation while preserving varietal identity.

Intra-cultivar variability refers to stable and recognizable phenotypic and genetic differences among clones or populations belonging to the same cultivar. These differences may result from long-term selection under contrasting agroecological conditions or from spontaneous mutations that have proven viable and adaptive. A well-known example is Tempranillo in Spain, for which more than 50 commercial clones are available as a result of its historical cultivation across a broad geographic range [5]. In this regard, previous studies have shown substantial intra-cultivar variation in traits relevant to drought response. In Tempranillo, for instance, significant differences among clones have been reported for physiological variables associated with water use and drought performance [6], while mesophyll conductance, respiration rate, and photosynthetic capacity have been identified as major determinants of clonal differences in water-use efficiency [7]. These findings suggest that intra-cultivar diversity may provide valuable adaptive resources for viticulture under climate change.

For cv. País, however, the available information remains very limited. Although this cultivar is generally considered tolerant to water deficit, only a few studies have evaluated the effects of drought on its productivity and fruit quality [8], and virtually none have addressed the physiological mechanisms underlying this apparent tolerance. To our knowledge, no study has specifically examined intra-cultivar variability in traits associated with drought-response strategy in cv. País. This knowledge gap is particularly relevant because drought adaptation in grapevine is a complex trait that integrates water use, gas exchange, growth, and yield responses across multiple organizational scales [9].

A widely used framework for describing drought responses in grapevine has been the distinction between iso- and anisohydric behaviour, referring to genotypes that show stronger or weaker regulation of plant water status through stomatal control, respectively [10,11]. More recently, this distinction has been refined through definitions based on daily water potential homeostasis or stomatal closure thresholds [12]. Nevertheless, the biological mechanisms underlying these water-relation strategies extend beyond stomatal regulation alone and remain incompletely understood, as they depend on both the conceptual framework adopted and the environmental conditions under which plants are evaluated [13]. Moreover, despite the large number of studies published on grapevine drought responses during the last two decades, it remains unclear whether a more isohydric or anisohydric behaviour confers greater adaptive value under water deficit [9]. This

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uncertainty supports the need to focus on measurable physiological traits that provide a more robust basis for comparing genotypes and predicting performance under specific environmental scenarios. In this context, hydraulic and water-use traits have been proposed as useful descriptors of drought-response strategy. According to [9], key traits include maximum transpiration rate at the whole-plant level, stomatal regulation in response to declining plant or soil water status, and other integrative variables related to plant water use.

Taken together, these antecedents indicate that assessing intra-cultivar variability may represent a key strategy for improving the resilience of traditional viticultural systems under climate change, particularly in patrimonial cultivars such as País. At the same time, the complexity of drought-response traits and their strong interaction with environmental conditions demand robust experimental approaches capable of detecting subtle but meaningful physiological differences among genotypes. Based on the above, we hypothesized that clones originating from more arid zones would exhibit greater resistance to water deficit, reflected in improved plant water status, lower maximum stomatal conductance, and more efficient stomatal regulation than clones from less arid areas. Accordingly, the objective of this study was to characterize intra-cultivar variation in the drought-response strategy of *Vitis vinifera* L. cv. País from contrasting geographic origins by quantifying: (i) the relationships among substrate water availability, stem water potential, and stomatal conductance; (ii) the hydraulic thresholds associated with stomatal decline (P12, P50, and P88); and (iii) the recovery of plant water status and stomatal conductance after rewatering, using Cabernet Franc as a comparative reference.

2. Materials and Methods

Study Site and Plant Material

The study was conducted at the Limarí Campus of the University of La Serena (30°35′01″S, 71°11′40″W), in the Coquimbo Region of Chile. According to the Köppen–Geiger climate classification, the area has a semi-arid climate with winter rainfall (BSk), characterized by high solar radiation, semi-arid conditions, and marked thermal seasonality [14]. Within the Limarí basin, specifically in the Cordillera Media Elqui–Limarí climatic unit, the CIREN Observatory reports an average maximum temperature of 23.5 °C in January and an average minimum temperature of -5 °C in June, reflecting the strong winter thermal variability typical of the Norte Chico region of Chile (CIREN, 2024). In this basin, the influence of the Pacific Ocean and the Coastal Range moderates daily thermal amplitude and contributes to relatively stable winter conditions, whereas relative humidity is partly influenced by the proximity of the Limarí River (Dirección Meteorológica de Chile, 2025).

The experiment lasted five weeks and was conducted using two-year-old *Vitis vinifera* L. cv. País plants from three geographic origins: Arica, Huasco, and Limarí (Figure 1). The provenance site Arica corresponds to a cold desert climate with summer rainfall and a hyper-arid classification, whereas Huasco and Limarí correspond to a semi-arid climate with winter rainfall, with aridity categories of arid and semi-arid, respectively [14,15]. The plant material was provided by INIA Intihuasi, which has established a collection of cv. País clones through prospection of vineyards in northern Chile in collaboration with local growers and regional INIA centers. To ensure varietal identity, the plant material was genetically characterized by study group using single nucleotide polymorphism (SNP) markers and genotyped through the Gen_SNP_1274 platform [16,17].

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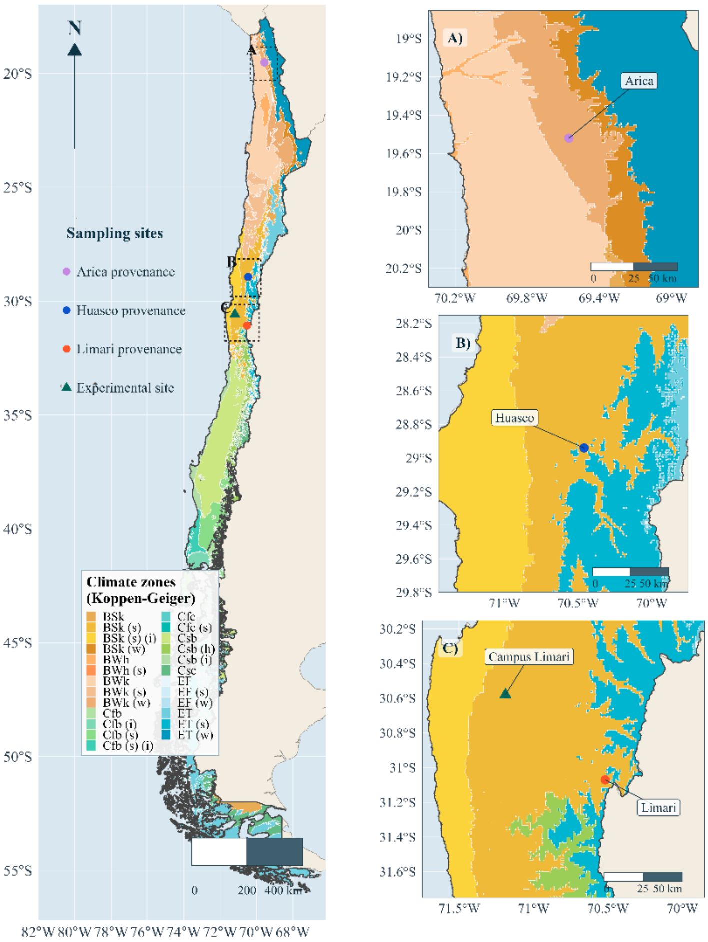

Figure 1. Geographic location of the sampling sites and climatic context of the provenances used in this study. The left panel shows the distribution of Köppen-Geiger climate zones across continental Chile, with the locations of the three provenance origins indicated: Arica (19.52° S, 69.57° W; 933 m a.s.l.), Huasco (28.94° S, 70.46° W; 1716 m a.s.l.), and Limarí (31.07° S, 70.52° W; 742 m a.s.l.). The experimental site at Campus Limarí is also shown (30.58° S, 71.19° W; 315 m a.s.l.). Panels A-C provide enlarged views of the areas corresponding to each provenance: (A) Arica, (B) Huasco, and (C) Limari, with the Campus Limari site included in the southern inset.

During the winter of 2025, ten plants per origin were selected and subjected to preventive sulfur and potassium soap applications. Plants were then transplanted into 11-L pots containing a homogeneous substrate composed of a 1:1 mixture of peat and agricultural soil collected from the study site. A 5-cm layer of perlite was placed at the bottom of each pot to improve drainage. At transplanting, plants were pruned to three buds, fertilized with 10 g plant^{-1} of granular NovaTec® Classic 12-8-16(+3+TE), and maintained under irrigation as needed until September 29, when the irrigation treatments were initiated.

Experimental Design

Two fixed factors were evaluated in a factorial arrangement: (i) geographic origin (GO), with three levels (Arica, Huasco, and Limarí), plus a control represented by the cultivar Cabernet Franc, known for its high stomatal tolerance to water deficit [18]; and (ii) water availability (WA), with two levels: well-watered (WW) and water deficit (WD). Treatments were arranged in a completely randomized design. Five replicates were established by treatment combination, resulting in a total of 40 experimental units (4 × 2 × 5). Each experimental unit consisted of a single plant grown individually in an 11-L pot containing a homogeneous substrate.

Pots assigned to the well-watered treatment were irrigated to saturation, defined as visible drainage, every two days. In contrast, pots assigned to the water-deficit treatment received no irrigation throughout the experimental period.

Measured Variables

Leaf Area

Leaf area (LA, m^2) was estimated at the beginning of the experiment and during the consecutive three weeks using a non-destructive allometric model based on projected plant area. For model calibration, actual leaf area (cm^2) was measured, and lateral photographs of each plant were taken simultaneously (Figure S1). Images were subsequently analyzed with ImageJ to quantify green projected area. Both variables were related through linear regression, yielding a model that allowed leaf area to be estimated from photographs throughout the experimental period.

Relative Pot Water Content

Before the onset of the water-deficit treatment, all pots were irrigated to saturation, allowed to drain until no further water flow was observed, and then weighed to determine maximum pot weight (M_max; g). This value was considered equivalent to pot capacity, that is, maximum water availability (Supplementary Table S1). During the experiment, pot weight (M) was recorded using a balance with a nominal capacity of 20 kg and a precision of 0.02% (equivalent to 4 g). Relative pot water content (RPWC) was then estimated as $\mathrm{RPWC} = {}^{M}/_{M_{max}}$.

Stem Water Potential, Stomatal Conductance and Sensitivity of Stomatal Conductance to Water Deficit

Stem water potential (Ψ, MPa) was measured weekly from the onset of the water-deficit treatment onward, around solar noon (13:30 h local time), on one fully expanded leaf per plant using a Scholander-type pressure chamber (Pump-up Chamber, PMS Instruments, USA). Leaves were enclosed in aluminized plastic bags for 1 h before measurement [19].

At the same frequency and within the same time window, stomatal conductance (mmol m^{-2} s^{-1}) was measured using a steady-state porometer (SC-1 Leaf Porometer, METER, USA) on two fully expanded, radiation-exposed leaves per plant.

To infer stomatal conductance behavior, stem water potential values corresponding to reduction of 12%, 50%, and 88% of maximum stomatal conductance were derived analytically from the fitted parameters for each provenance. Standard errors and confidence intervals for these derived thresholds were obtained using the delta method applied to the covariance matrix of the nonlinear parameters. Pairwise differences among provenance-specific threshold estimates were then evaluated using approximate Wald tests, and Holm-adjusted p-values were used to identify statistically supported differences among provenances.

Recovery Capacity

During the sixth week of the experiment, irrigation was restored in plants assigned to the water-deficit treatment. These plants were irrigated on two consecutive days until pot weight reached between 93 and 97% of maximum pot weight. On the third day, stem water potential and stomatal conductance were measured as described above.

Environmental Conditions

Meteorological characterization of the study site was based on records from the Escuela Agrícola Ovalle agrometeorological station (30.58°S, 71.18°W; 314 m a.s.l.), part of the Agroclima Network, located approximately 1 km in a straight line from the experimental site.

Data Analysis

All analyses were performed in R [20]. Repeated-measures responses were analyzed using linear mixed-effects models fitted with the lmerTest package [21], which extends lme4 to provide inferential testing for mixed models (Bates et al., 2015). Provenance, irrigation treatment, week, and their interactions were treated as fixed effects, whereas experimental unit was included as a random intercept. Type III tests were extracted for the fixed effects. Residual normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test implemented in the car package [22]. When heteroscedasticity was detected, analyses were refitted using variance-structured mixed models in nlme with a varIdent residual variance function, allowing treatment- or time-specific residual variances while preserving the repeated-measures structure [23]. Post hoc inference was based on estimated marginal means obtained with emmeans [24]. Compact letter displays were generated using multcomp [25]. Pairwise comparisons were performed with Tukey adjustment at $\alpha = 0.05$ whenever the contrast family was compatible with Tukey control; otherwise, Sidak correction was applied through emmeans.

Regression analyses were conducted separately for each provenance. Nonlinear models (power [Eq. 1], logistic [Eq. 2] and Weibull [Eq. 3]) were fitted using minpack.lm [26], and the best model was selected according to the Akaike information criterion (AIC). Confidence bands for nonlinear fits were derived from the variance–covariance matrix of the estimated nonlinear parameters using a first-order delta-method approximation. Graphs were produced with ggplot2 [27], and data manipulation was performed mainly with dplyr and tidyr [28].

$$\text{Power: } \mathrm{y} = \mathrm{a}\,\mathrm{x}^{\mathrm{b}} + \mathrm{c} \tag{1}$$

$$\text{Logistic: } y = \frac{a}{1 + \exp\left(-\frac{x-b}{c}\right)} \tag{2}$$

$$\text{Weibull: } y = K_{max}\exp\left[-\ln(2)\left(\frac{x}{P_{50}}\right)^{s}\right] \tag{3}$$

The series comparison presented in the regression figures corresponds to an extra sum-of-squares F-test. Under this framework, the reduced model consisted of a single common curve fitted across all provenances, whereas the full model consisted of provenance-specific curves. A significant overall series comparison indicated that provenance-specific curves explained significantly more residual variation than a shared curve, implying that at least one provenance differed in the form or position of its fitted response. Provenance differences were identified using Holm-adjusted p-values at $\alpha = 0.05$. In addition, provenance-specific nonlinear parameter estimates were compared pairwise within each relationship and model family using approximate Wald tests with Holm adjustment.

3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Meteorological and Water Deficit Conditions

During the experimental period, the mean daily air temperature was 15.1 ± 1.83 °C, mean daily relative humidity was 72.1 ± 9.3%, and mean daily solar radiation was 19.9 ± 5.7 MJ m^{-2}.

Relative pot water content remained high and comparatively stable in well-watered plants throughout the experimental period, generally oscillating around 0.95-1.02 across provenances. By contrast, water-deficit plants showed a progressive decline from values close to 0.95 at the beginning of the stress period to approximately 0.61-0.65 at the end of the experiment. This depletion followed a similar temporal trajectory among provenances, although Limarí tended to retain slightly higher relative pot water content under water deficit, whereas CF reached the lowest values during the final measurement dates (Figure 2). These trends in water acquisition capacity do not appear to be associated with leaf area, as this variable did not differ significantly between treatments, and no interaction between provenance and irrigation treatment was detected (Figure S2).

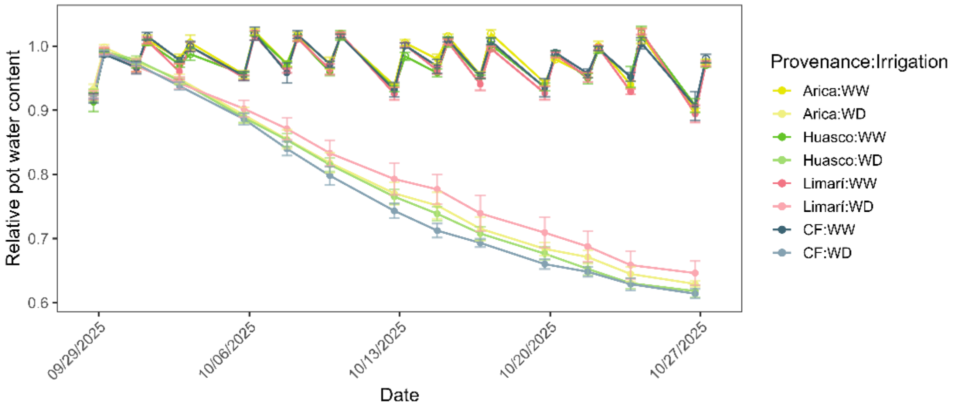

Figure 2. Relative pot water content of well-watered (WW; open symbols) and water-deficit (WD; filled symbols) plants from the Arica, Huasco, Limarí and CF provenances across measurement dates. Values are presented as mean ± SE (*n* = 5).

3.2. Water Potential Regulation and Stomatal Behavior

Nonlinear fits revealed significant provenance-specific relationships between relative pot water content and both stem water potential and stomatal conductance (global series comparison, $p < 0.001$ in both cases; Table S1). As substrate water availability declined, stem water potential became more negative in all provenances (Figure 3A), but the CF series was displaced toward lower values and differed from Arica, Huasco, and Limarí (Holm-adjusted $p < 0.001$ in all three contrasts), whereas Arica and Limarí also differed from each other ($p = 0.015$). Stomatal conductance likewise declined nonlinearly with decreasing relative pot water content ($p < 0.001$), and CF displayed a distinct response relative to Arica ($p = 0.006$), Huasco ($p = 0.001$), and Limarí ($p < 0.001$), consistent with its higher fitted asymptotic conductance (Figure 3B).

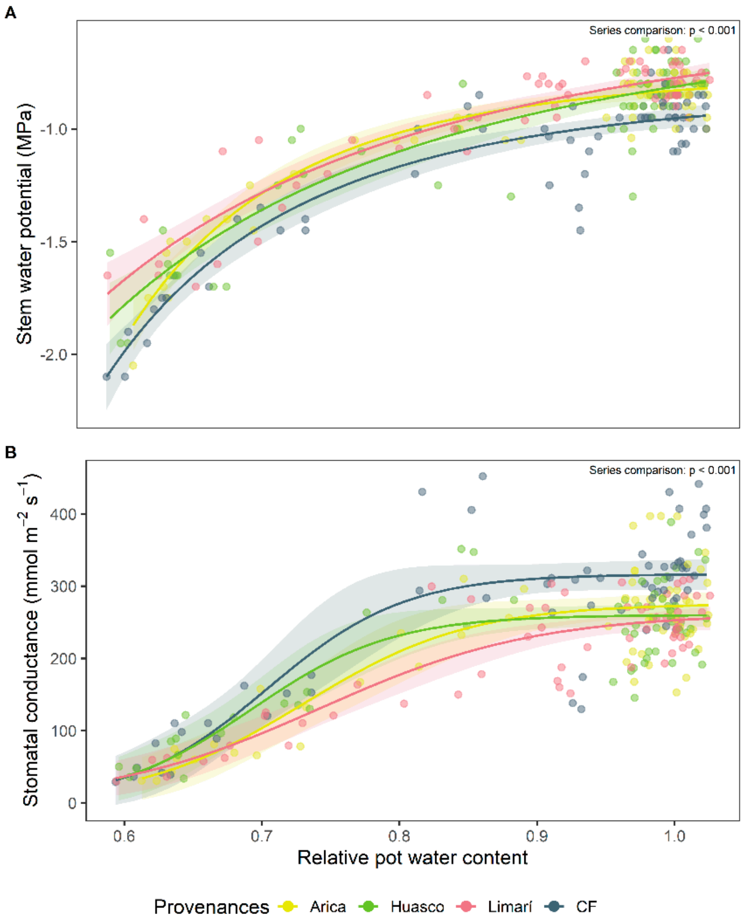

Figure 3. Nonlinear relationships between relative pot water content and stem water potential (A) and between relative pot water content and stomatal conductance (B) for the four provenances. Points are individual values, solid lines represent fitted response curves (power-model and logistic regressions for panel A and B, respectively) for each provenance, and shaded areas indicate the 95% confidence intervals. The series-comparison p-values correspond to extra sum-of-squares F-tests comparing a common curve with provenance-specific curves.

The relationship between stomatal conductance and stem water potential was also provenance-dependent (global series comparison, $p < 0.001$; Table S1), and all pairwise series contrasts remained significant after Holm correction ($p = 0.031$ to $p < 0.001$). Across the increasing water-stress gradient, CF maintained the highest stomatal conductance, whereas Huasco and Limarí exhibited earlier and steeper declines (Figure 4A). Consistently, maximum stomatal conductance measured at stem water potentials >= -1 MPa differed among provenances (provenance effect, $p < 0.001$), with CF showing the highest marginal mean (333.9 mmol m^{-2} s^{-1}) and forming a distinct post hoc group from Arica (275.4 mmol m^{-2} s^{-1}), Huasco (267.1 mmol m^{-2} s^{-1}), and Limarí (249.0 mmol m^{-2} s^{-1}), which did not differ from one another (Figure 4B).

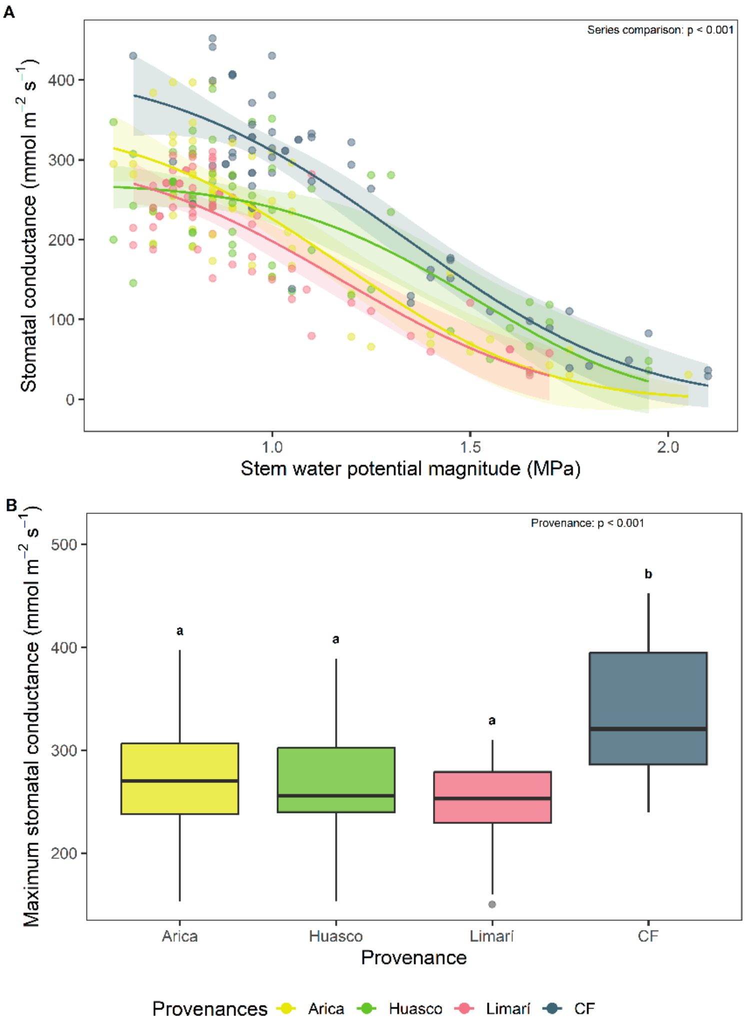

Figure 4. Relationship between stomatal conductance and stem water potential magnitude (A) and provenance differences in maximum stomatal conductance measured at stem water potentials greater than or equal to -1 MPa (B). In panel A, Points are individual values, solid lines represent fitted response curves (Weibull regressions) for each provenance, and shaded areas indicate the 95% confidence intervals. The series-comparison p-values correspond to extra sum-of-squares F-tests comparing a common curve with provenance-specific curves. The series-comparison p-value is derived from an extra sum-of-squares F-test contrasting a common Weibull curve with provenance-specific curves. In panel B letters denote post hoc groupings from multiplicity-adjusted pairwise comparisons among provenance estimated marginal means.

Hydraulic threshold estimates derived from stomatal conductance vulnerability curves (Weibull and logistic regression) showed the clearest provenance separation at P50 (Figure 5; Tables S2 and 3). Huasco displayed the most negative P50 values in both model families (approximately -1.47 to -1.48 MPa), whereas Arica and Limarí reached 50% stomatal reduction at less negative potentials (approximately -1.16 to -1.18 MPa), with CF showing intermediate values near -1.33 MPa. Under the logistic model, the contrasts Arica-Huasco and Huasco-Limarí were significant after Holm adjustment (both $p = 0.045$), whereas the same contrasts were marginal under the Weibull model ($p = 0.051$ and $p = 0.053$, respectively). In contrast, P12 and P88 estimates largely overlapped among provenances, and no pairwise differences remained significant after multiplicity correction (Figure 5).

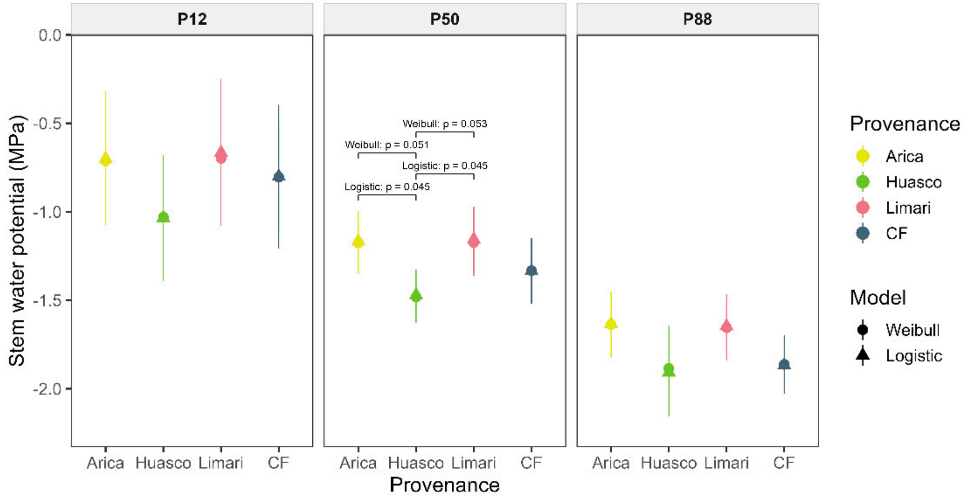

Figure 5. Provenance-specific hydraulic thresholds derived from stomatal conductance vulnerability curves, expressed as P12, P50 and P88, corresponding to reduction in 12%, 50% and 88% of maximum stomatal conductance, respectively. Points are threshold estimates and vertical bars are 95% confidence intervals; circles and triangles distinguish Weibull and logistic models. Horizontal brackets identify pairwise provenance contrasts displayed for each threshold, and the associated labels report adjusted p-values. Post hoc comparisons among provenances were performed separately within each threshold and model family using Holm-corrected pairwise tests.

Repeated-measures analysis indicated that the three-way interaction among provenance, irrigation treatment, and week was not significant (Provenance × Irrigation × Week, $p = 0.657$), nor was the Provenance × Irrigation interaction ($p = 0.236$). By contrast, provenance, week, and the Provenance × Week interaction significantly affected both stem water potential and stomatal conductance (all $p < 0.001$). Stem water potential (Figure 6A) declined progressively across weeks in all provenances, but this decline was strongest in CF, which became more negative from week 2 onward and reached the lowest marginal mean at week 4 (-1.44 MPa). Stomatal conductance (Figure 6B) also decreased over time, with CF showing the highest values at the beginning of the experiment, peaking at week 1 (390.1 mmol m^{-2} s^{-1}), and converging with the remaining provenances under more severe stress by weeks 3 and 4.

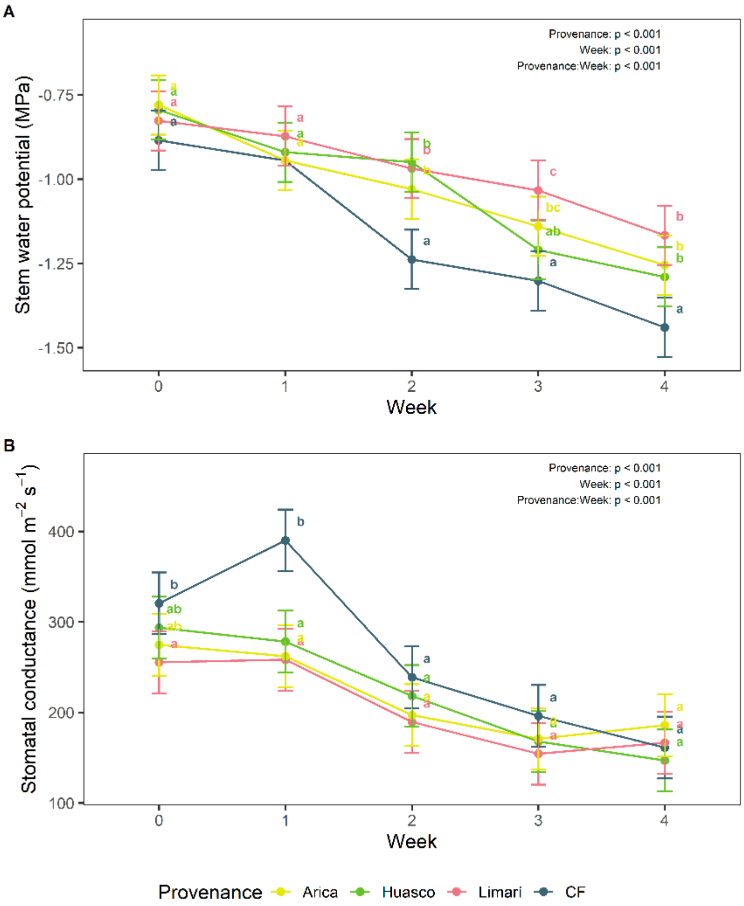

Figure 6. Estimated marginal means of stem water potential (A) and stomatal conductance (B) for each provenance across the experimental weeks. Points represent estimated means and vertical bars indicate 95% confidence intervals. The p-values shown in each panel correspond to the effects of provenance, week and the provenance-by-week interaction in the repeated-measures model. Letters indicate compact letter displays obtained from adjusted post hoc pairwise contrasts among provenance means within each week.

3.3. Recovery Capacity of Water Potential and Stomatal Conductance After Irrigation Restitution

During recovery, both stem water potential (Figure 7A, B) and stomatal conductance (Figure 7 C, D) were significantly affected by treatment, recovery stage, and their interaction (all $p < 0.001$). Before irrigation restoration, well-watered plants had substantially less negative stem water potential (-0.73 to -0.95 MPa) and higher stomatal conductance (247.3-322.7 mmol m^{-2} s^{-1}) than water-deficit plants (-1.60 to -1.93 MPa and 38.9-62.4 mmol m^{-2} s^{-1}, respectively). After irrigation restoration, water-deficit plants partially recovered, particularly in stem water potential, with Limarí and Arica approaching -0.81 and -0.89 MPa, respectively, whereas CF remained the most negative (-1.24 MPa). Stomatal conductance also increased markedly after rewatering in all stressed groups (187.7-201.4 mmol m^{-2} s^{-1}), yet values generally remained below those of the best-performing well-watered treatments, especially Arica:WW and CF:WW (Figure 7).

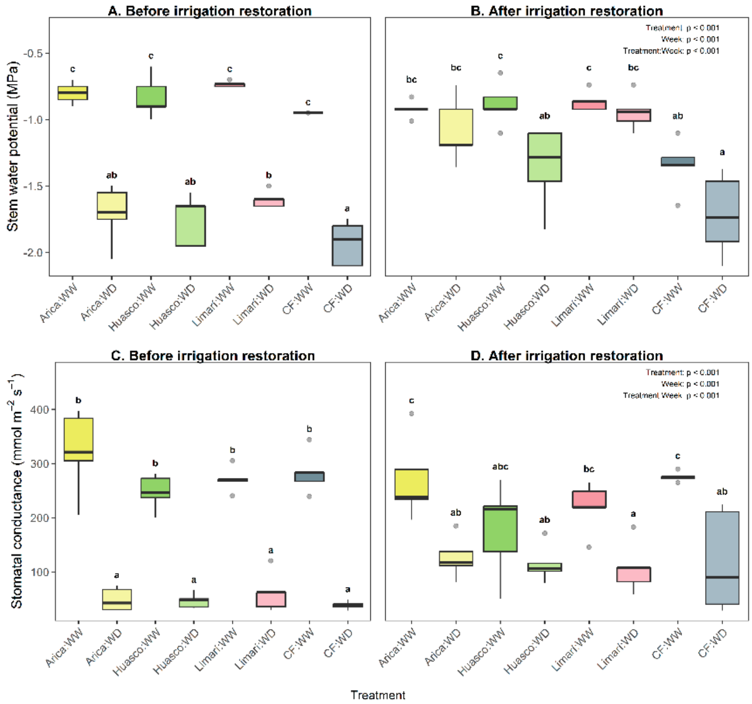

Figure 7. Recovery responses shown as boxplots for stem water potential before (A) and after (B) irrigation restoration and for stomatal conductance before (C) and after (D) irrigation restoration, across provenance-by-irrigation combinations. The p-values correspond to the effects of treatment, recovery stage and their interaction in the recovery analysis. Letters denote post hoc groupings from adjusted pairwise comparisons among provenance-by-irrigation combinations within each recovery stage.

4. Discussion

The progressive decrease in relative pot water content from values close to 0.95 at the onset of the treatment to approximately 0.61–0.65 at the end of the experiment indicates that water deficit was imposed gradually and consistently across provenances. This is relevant because the physiological differences detected among materials were expressed along a shared dehydration trajectory (Figure 2) rather than under abrupt desiccation. In grapevine, stem water potential usually operates within a broad interval of approximately −0.3 to −2.0 MPa, while regulated deficit irrigation commonly targets values around −1.2 to −1.4 MPa; more severe drought effects become increasingly likely below about −1.6 MPa, when stomatal restriction intensifies and hydraulic risk increases [9]. In the present study, water-deficit plants reached −1.60 to −1.93 MPa before rewatering, indicating that the final phase of the assay extended beyond moderate stress and approached the severe range for grapevine.

Against that background, cv. País showed a comparatively conservative stomatal behavior relative to Cabernet Franc, reported as an highly anisohydric cultivar [18]. Cabernet Franc maintained the highest stomatal conductance across the dehydration gradient and the highest maximum stomatal conductance (gs) under mild stress, with a marginal mean of 333.9 mmol m^{-2} s^{-1} at Ψstem ≥ −1 MPa and a peak of 390.1 mmol m^{-2} s^{-1} at week 1. By contrast, the País provenances showed lower maximum values, ranging from 249.0 mmol m^{-2} s^{-1} in Limarí to 275.4 mmol m^{-2} s^{-1} in Arica. These values are consistent with the upper field range reported for Grenache clones by [29], who found gs values between 0.079 and 0.285 mol m^{-2} s^{-1}, and with the broader pot-based range reported by [30], where gs ranged from 0.012 to 0.590 mol m^{-2} s^{-1}. Thus, the present experiment falls within the physiological domain described for grapevine drought studies, but the lower maximum stomatal conductance of País relative to Cabernet Franc supports the interpretation of a more conservative water-use phenotype. This behavior, and considering the higher maximum stomatal conductance observed in CF (Figure 4B), is consistent with a lower water-use capacity, reflected in the slower decline in relative pot water content (Figure 2) in País compared with CF, despite the fact that leaf area did not differ significantly between them (Figure S2).

Stem water potential declined progressively in all provenances (Figure 6A), but this decline was strongest in Cabernet Franc, which reached the lowest marginal mean at week 4 (−1.44 MPa). At the same time, stomatal conductance (Figure 6B) in Cabernet Franc remained comparatively high during the initial phase of the experiment and only converged with the remaining provenances under stronger stress. This suggests that Cabernet Franc maintained higher transpirational activity during early substrate drying, whereas País reduced stomatal opening sooner or at lower absolute conductance values. Physiologically, this agrees with the framework proposed by [9], who noted that grapevines often reduce gs strongly within the same water-potential range used in deficit irrigation management, and that non-stomatal limitations become increasingly important when gs falls below about 50 mmol m^{-2} s^{-1}. In the present study, the most stressed plants reached 38.9–62.4 mmol m^{-2} s^{-1} before rewatering, indicating that at least part of the response occurred near the transition between strong stomatal limitation and more severe physiological restriction.

The original hypothesis, however, was only partially supported, because the aridity gradient of origin did not translate into a simple ranking of drought resistance. The clearest provenance differentiation appeared at P50 (Figure 5), where Huasco reached 50% stomatal reduction at approximately −1.47 to −1.48 MPa, whereas Arica and Limarí reached the same level of closure at markedly less negative stem water potentials, approximately −1.16 to −1.18 MPa; Cabernet Franc was intermediate, near −1.33 MPa. Therefore, Huasco tolerated a decline of roughly 0.30 MPa beyond Arica and Limarí before reaching midpoint stomatal closure being Huasco close to the lower boundary of the deficit range that [9] described as agronomically relevant for grapevine water restriction. At the same time, P12 and P88 largely overlapped among provenances, suggesting that the central portion of the stomatal response curve was more informative than its early or near-terminal sections. This agrees with the argument of [9] that midpoint thresholds are often more robust than extreme closure thresholds, where asymptotic behavior increases uncertainty.

The provenance pattern also deserves a more nuanced interpretation because Huasco and Limarí showed earlier and steeper declines in the gs–Ψstem relationship (Figure 4A), yet Huasco displayed the most negative P50. This apparent contrast indicates that provenance effects were expressed through several dimensions of the response curve, including maximum conductance, curve steepness, and the water-potential position of midpoint closure, rather than through a single descriptor. In practice, Huasco did not necessarily maintain the highest conductance across the entire drought trajectory, but it did maintain 50% of its maximum conductance to more negative stem water potentials than the other País provenances. This distinction is important because it avoids reducing drought behavior to a simple categorical label [13]. As emphasized by [9], grapevine drought response is better interpreted through measurable traits and fitted response functions than through overly simplified classifications. Under this framework, País can be described here as a cultivar with an overall conservative stomatal strategy relative to Cabernet Franc, but also as one that contains meaningful provenance-dependent variation in how stomatal decline is deployed during progressive drying.

The existence of intra-cultivar variation in País is consistent with previous grapevine studies. [30] showed that under pot conditions nine Grenache genotypes covered a Ψstem range from −0.25 to −1.60 MPa and a gs range from 0.012 to 0.590 mol m^{-2} s^{-1}, with genotype-specific differences in both water relations and stomatal control. In field-grown Grenache, [29] likewise found differences among clones in seasonal Ψstem and gs, and reported ranges of −0.89 to −0.47 MPa in Ψstem and 0.079 to 0.285 mol m^{-2} s^{-1} in gs depending on season and genotype. [31] further summarized that intracultivar variability in WUEi reached about 30% in Grenache under field conditions and remained detectable under pot conditions. Taken together, those studies indicate that the magnitude of physiological differentiation observed here among País provenances is fully plausible within an intracultivar framework and should not be interpreted as mere experimental noise. Rather, the present work

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extends that evidence to a patrimonial cultivar for which mechanistic analyses of drought-response strategy have been largely lacking.

The present results are also consistent with earlier evidence from Chilean grapevine resources. [32], working with naturalized grapevine genotypes from arid and semi-arid northern Chile, identified both anisohydric and near-isohydric response patterns and found that drought tolerance varied substantially among genotypes under controlled stress. Although that study involved different plant material and a distinct experimental system, it supports the broader idea that northern Chilean grapevine resources harbor substantial physiological diversity in water-stress responses. By contrast, [33] found that dry-grown Cabernet Sauvignon vines differing in rooting depth maintained similar midday stem water potentials, around −0.89 and −0.81 MPa, while gs averaged 74 ± 9 and 104 ± 8 mmol m^{-2} s^{-1}. Compared with those values, the stressed plants in the present study reached more negative stem water potentials and similarly low or lower minimum gs, showing that the imposed drought was sufficiently intense to reveal provenance-dependent regulatory differences within País.

Before irrigation restitution (Figure 7), water-deficit plants showed Ψstem values between −1.60 and −1.93 MPa and gs between 38.9 and 62.4 mmol m^{-2} s^{-1}. After rewatering, stem water potential recovered markedly, especially in Limarí and Arica, which approached −0.81 and −0.89 MPa, respectively, whereas Cabernet Franc remained more negative at −1.24 MPa. Stomatal conductance also increased strongly after rewatering, reaching 187.7–201.4 mmol m^{-2} s^{-1} in previously stressed plants, but it generally remained below the highest well-watered values. This partial decoupling between hydraulic recovery and stomatal reopening is physiologically plausible and suggests that water status recovered faster than gas-exchange regulation. The recovered Ψstem values of Arica and Limarí are particularly notable because they are very close to the midday Ψs reported by [33] for functioning dry-grown Cabernet Sauvignon vines. These result suggest a better performance in mora isohydric provenances [34].

From an agronomic perspective, these findings are relevant because cv. País has long been associated with rainfed systems and patrimonial viticulture, yet it has been much less studied physiologically than modern commercial cultivars. [8], working on Negra Criolla, did not report stem water potential or stomatal conductance, but they did show that irrigation regime altered bunch mass and must composition, demonstrating that the País/Negra Criolla lineage is agronomically responsive to water supply. The present study therefore provides a physiological basis for future selection within País, while preserving varietal identity and patrimonial value. Accordingly, the current results should be interpreted as a first mechanistic screening rather than as definitive evidence of field performance. The next step should be to expand the number of provenances evaluated and to test whether the provenance differences identified here translate into differences in long-term productivity, water-use efficiency, and fruit quality under rainfed and deficit-irrigated vineyard conditions.

5. Conclusions

This study demonstrates that *Vitis vinifera* L. cv. País contains physiologically relevant intra-cultivar variation in drought-response strategy. Under progressive substrate drying, País behaved more conservatively than Cabernet Franc, as indicated by lower maximum stomatal conductance and a less extreme decline in stem water potential during much of the stress period. However, this conservative behavior was not homogeneous among provenances. Huasco showed the most negative P50, whereas Arica and Limarí reached midpoint stomatal closure at less negative stem water potentials, indicating that the clearest provenance differentiation occurred in the central portion of the stomatal vulnerability curve rather than at P12 or P88.

The results also show that drought response in País cannot be explained by source-site aridity alone. Instead, each provenance combined maximum stomatal conductance, threshold position, and recovery after rewatering in a distinct way. In practical terms, this suggests that provenance selection within País may offer a useful strategy for improving adaptation to water-limited viticulture while preserving cultivar identity. Because the present study was conducted under semi-controlled pot

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conditions and over a short drought cycle, these conclusions should now be validated under field conditions and linked to agronomic traits such as biomass production, yield, and fruit composition.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: title; Table S1: title; Video S1: title.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, M.G. and A.P.; methodology, M.G.; formal analysis, M.G. and M.P.; investigation, M.G.; resources, M.G. and N.V.; data curation, M.G., A.P., M.P.; writing—original draft preparation, M.G., A.P.; writing—review and editing, M.P., A.P., E.V., I.O. N.V., F.C. and C.F.; visualization, M.G., M.P., I.O., C.F. and F.C.; supervision, M.G.; project administration, M.G.; funding acquisition, M.G. All authors have read and agreed to the published version of the manuscript.

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