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# Seed germination and phenotypic responses to water restriction of *Beilschmiedia miersii* provenances

Carlos Magni[1], Paola Poch[1], Sergio Espinoza[2], Marco Yañez[3], Eduardo Martínez[1], Álvaro Promis[1], Gabriel Mancilla[4]

[1]Facultad de Ciencias Forestales y de la Conservación de la Naturaleza, Universidad de Chile, Santiago, Chile. crmagni@uchile.cl.
[2]Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, Talca, Chile. espinoza@ucm.cl
[3]Instituto de Investigación Interdisciplinaria, Vicerrectoría Académica,Universidad de Talca, Talca, Chile
[4]Centro del Agua para Zonas Áridas y Semiáridas de América Latina y el Caribe (CAZALAC).

## Abstract

We assessed the effect of seed provenance on seed germination traits, seedling growth, biomass allocation, presence of cotyledons, and survival responses of the endemic *Beilschmiedia miersii* cultivated under two water treatments (well- watered versus water stress, average pre-dawn plant water potentials of -0.9 and -4.5 MPa). Provenances from the southern range of the species had a better germination performance whereas the coastal and interior provenances were not able to survive in large number to the seedling stage. The provenance El Arbol, exhibited a higher survival, growth, presence of cotyledons, and biomass traits. This provenance also exhibited a higher presence of cotyledons in both watering treatments and an unaltered root to shoot ratio between the well- watered and the water stress treatments. We found important phenotypic variation for seed germination and seedling survival associated to the provenance origin, emphasizing the importance of a nursery-evaluation phase before the beginning of restoration projects at the landscape level.

Keywords: provenance origin, water restriction, restricted range species, Mediterranean-type ecosystems, recruitment, restoration

## Introduction, scope and main objectives

*Beilschmiedia miersii* (Gay) Kosterm is an endemic and threatened recalcitrant-seeded tree species with a very restricted distribution range (Henriquez and Simonetti 2001) and the largest seeds among Mediterranean flora (Fuentes et al. 1986). Its overall geographical range extends no more than 200 km from coastal areas (32° S) and altitudes close to the sea level, to interior areas (34° S) with altitudes close to 1,200 m.a.s.l (Donoso 1998). *B. miersii* is a relict species (Cabrera 1959) that has been typically restricted to humid and shaded environments (Becerra et al. 2004), but current populations correspond to remnants of communities developed under different environmental conditions. In this context, given the contrasting environmental conditions within the limited area where *B. miersii* currently develops, it is expected phenotypic variation in seed germination and seedling responses, especially to environmental stresses such as drought. In field experiments with *B. miersii* it was found that seedling survival improved with irrigation, and the response depended on the site canopy conditions and the presence of leaf litter (Kremer et al. 2019), whereas, under controlled conditions, seedling survival was not affected by irrigation (Becerra et al. 2004). However, while there is concern about the restoration of *B. miersii*, there is still little knowledge about the provenance and phenotypic variation of different seed sources to stresses such as drought at the seedling stage. The aim of this study was to analyze the variation for seed germination and the phenotypic responses to water restriction of *B. miersii* provenances under controlled conditions.

## Methodology/approach

We used seeds from four provenance origins from coastal and inland areas of Central Chile and one plantation established with seeds from an interior provenance (Table 1). From February to early April 2013, ripen seeds were collected directly from 40 mother trees and stored at 4°C under similar conditions until use. ISTA standards were followed to clean seeds (ISTA 2006). In April 2013 the seeds were soaked in tap water for 24 h, and those that floated and damaged discarded. Approximately sixteen viable seeds from each of 40 mother trees (i.e., a total of 3,168 seeds) were sown in 150-mL pots filled with a mixture of composted bark, local topsoil, and sand (1:1:1 v). Then, they were cultured under similar ambient conditions for 12 months until early April 2014 under daily irrigation. No fertilizer was added to the growing substrate. Germination was recorded daily on those seeds with emerged cotyledons. The germination capacity (GC) was obtained as the proportion between the germinated seeds and the total seeds sown. The maximum value of Czabator (MV) was obtained as the maximum quotient derived from all the cumulative full-seed germination percentages on any day divided by the number of days to reach these percentages (Czabator 1962). We also determined the germinative energy (GE), as the accumulated percentage of germination on the day that MV occurs, and the energy period (EP), as the number of days in which MV occurs.

The watering experiment was established in April 2014 with 1-year-old seedlings, and it was arranged following a split-plot in an incomplete block design with 11 replicates. Two watering treatments were the whole plot treatment (well-watered (WW) and water stress (WS)), and the provenances were the split-plot treatment. Watering regimes were defined based on pre-dawn plant water potentials ($\psi_{pd}$) measured by using a Scholander pressure chamber (PMS Instrument, Albany, USA). In the WW treatment, seedlings were watered daily until reaching an average $\psi_{pd}$ of -0.9 MPa. In the WS treatment, water was withheld from April 11th to May 6th 2014 (i.e., one 26-days cycle of water withholding) and seedlings reached an average $\psi_{pd}$ of -4.5 MPa. The provenances El Pobre, Longotoma, and Antumapu were excluded from this analysis due to the low number of germinated seedlings. Provenances Cantillana and El Arbol were represented with 7 and 11 mother trees (synonym families), respectively, and the total number of seedlings in this phase was 1,265. Seedling height (H, cm), root collar diameter (D, mm), survival (SUR, %), and the presence of cotyledons in each seedling (COT, %) were measured after the watering treatments had finished. SUR and COT were assessed as categorical variables. Afterward, cotyledons were extracted from each seedling and weighed at ±0.01 (Wcot, g). Finally, all seedlings were harvested and oven-dried at 65°C until constant weight to obtain the dry weight fractions of roots (RDW), leaves (LDW), stems (SDW) (±0.01, g). The total dry weight (TDW) was derived as RDW+LDW+SDW, and the root:shoot ratio (RSR) was derived as RSR = RDW/(SDW+LDW).

Seed germination traits were analyzed using the full data set (n = 3,168 sown seeds) of the four provenances and the plantation, and the lineal mixed model presented in Equation 1. Otherwise, survival, growth, cotyledons, and biomass traits were analyzed using a dataset containing seedlings from provenances Cantillana and El Arbol (n = 1,265 seedlings), and the lineal mixed model presented in Equation 2. We examined the assumptions for normality and homogeneity of variance required for the analysis of variance and used Box-Cox transformation when necessary (Box and Cox 1964).

$$Y= \mu + R + P + R\times P + M(P) + R\times M(P) + e \quad (1)$$

$$Y= \mu + W + R + e_1 + P + M(P) + W\times P + W\times M(P) + e_2 \quad (2)$$

where, Y is the observed phenotypic value, μ is the overall mean, W is the fixed effect of watering regime, R is the fixed effect of replicate, P is the fixed effect of provenance, M(P) is the random effect of the mother tree nested within the provenance. In Equation 1, e represents the experimental random error. In Equation 2, $e_1$ is the error associated with the whole plot, and $e_2$ is the error associated with the split-plot. Mean comparisons were made using the Tukey test at $P \leq 0.05$.

**Table 1:** Location and climate for the provenances and plantation of *B. miersii* under study

| Provenance | Mother trees | Location | Latitude (X UTM) | Longitude (Y UTM) | Altitude (m.a.s.l) | MAP | MAT | De Martonne aridity index |
|---|---|---|---|---|---|---|---|---|
| **El Pobre** | 3 | Coastal | 283178 | 6431833 | 300 | 150 | 14.6 | 6.1 |
| **Longotoma** | 10 | | 277436 | 6415907 | 15 | 300 | 14.2 | 12.3 |
| **Antumapu*** | 3 | Interior | 348552 | 6284350 | 610 | 397 | 20.5 | 13.0 |
| **Cantillana** | 12 | | 321221 | 6256492 | 600 | 439 | 16.0 | 16.8 |
| **El Arbol** | 12 | | 313852 | 6252048 | 800 | 502 | 13.1 | 21.7 |

MAP = Mean Annual Precipitation (mm), MAT = Mean Annual Temperature (°C). The De Martonne aridity index was estimated as MAP/(MAT + 10). The higher the index, the lower the aridity. *This corresponds to a plantation established with seed collected in an interior provenance.

## Results

In the germination experiment, provenance El Arbol exhibited the highest GC, GE, and MV but no differences were observed for EP in any of the provenances under study (Table 2). In the water treatments experiment, the provenance El Arbol exhibited the highest D, H, SUR, SDW, LDW, RDW, TDW, COT, and Wcot (Table 3). Provenances also varied by watering treatment in RSR and COT. The Provenance Cantillana had a high RSR in the WW treatment, but this was reduced in the WS treatment, while RSR in the provenance El Arbol was similar in both treatments. Moreover, RSR in Cantillana provenance was higher than El Arbol provenance in the WW treatment but lower in the WS treatment (Fig. 1a). The provenance El Arbol had a higher percentage of cotyledons (COT) than Cantillana in both watering treatments (Fig. 1b), but in both provenances COT was significantly reduced by the WS treatment.

**Table 2:** Means and standard error for the seed germination parameters by provenance of *B. miersii*

| Trait | Provenance | | | | |
|---|---|---|---|---|---|
| | El Pobre | Longotoma | Antumapu | Cantillana | El Arbol |
| **Germinative capacity (GC, %)** | 30.0 ± 8.9b | 34.4 ± 8.1b | 48.4 ± 12.3b | 41.8 ± 2.9b | 80.4 ± 3.4a |
| **Germinative energy (GE, %)** | 27.2 ± 6.1b | 26.7 ± 9.4b | 43.3 ± 12.1b | 32.0 ± 3.5b | 62.8 ± 6.1a |
| **Maximum value (MV, % per day)** | 0.11 ± 0.02b | 0.17 ± 0.06ab | 0.18 ± 0.04ab | 0.23 ± 0.03ab | 0.40 ± 0.03a |
| **Energy period (EP, days)** | 246 ± 1a | 206 ± 50a | 246 ± 1a | 145 ± 12a | 172 ± 16a |

Different lowercase letters indicate significant differences among provenances at P ≤ 0.05.

**Table 3:** Main effects in growth, survival, and biomass parameters tested through 2-Way ANOVA using watering treatment (W) and provenance (P) as fixed factors and Family (F) as a random nested factor in provenance (F(P)). Statistically significant values (P ≤ 0.05) are in bold

| Trait | Water treatment | | Provenance | | Main effects of factors and interactions | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Well-watered | Water stress | Cantillana | El Árbol | Watering | Provenance | W×P | F(P) | W×F(P) |
| **D** | 4.55 ± 0.11 | 3.94 ± 0.08 | 4.05 ± 0.15 | 4.37 ± 0.08 | **<0.000** | **0.009** | 0.153 | 0.151 | 0.633 |
| **H** | 14.02 ± 0.52 | 12.31 ± 0.57 | 12.24 ± 0.71 | 13.72 ± 0.46 | **0.002** | **0.026** | 0.803 | **0.030** | 0.296 |
| **SUR** | 70.73 ± 3.25 | 52.85 ± 2.73 | 50.17 ± 4.43 | 68.45 ± 2.34 | **<0.000** | **0.003** | 0.732 | **0.001** | 0.980 |
| **SDW** | 0.73 ± 0.04 | 0.64 ± 0.03 | 0.59 ± 0.04 | 0.74 ± 0.03 | 0.142 | **0.001** | 0.056 | 0.270 | 0.055 |
| **LDW** | 1.00 ± 0.06 | 0.88 ± 0.06 | 0.81 ± 0.08 | 1.01 ± 0.05 | 0.073 | **0.017** | 0.162 | 0.082 | 0.065 |
| **RDW** | 1.23 ± 0.07 | 0.98 ± 0.06 | 0.93 ± 0.08 | 1.21 ± 0.06 | **0.003** | **0.003** | 0.254 | **0.013** | 0.414 |
| **TDW** | 2.98 ± 0.18 | 2.51 ± 0.15 | 2.33 ± 0.20 | 2.98 ± 0.14 | **0.025** | **0.002** | 0.121 | **0.044** | 0.089 |
| **RSR** | 0.73 ± 0.02 | 0.66 ± 0.02 | 0.70 ± 0.02 | 0.69 ± 0.02 | **0.026** | 0.766 | **0.019** | 0.222 | 0.922 |
| **COT** | 69.48 ± 3.17 | 62.17 ± 2.85 | 56.11 ± 4.16 | 71.08 ± 2.34 | **0.023** | **0.001** | **0.034** | **0.008** | 0.982 |
| **Wcot** | 2.83 ± 0.18 | 2.71 ± 0.17 | 2.18 ± 0.22 | 3.07 ± 0.14 | 0.532 | **0.008** | 0.070 | **0.016** | 0.619 |

D = root collar diameter, H = height, SUR = survival, SDW = stem dry weight, LDW = leaves dry weight, RDW = root dry weight, TDW = total dry weight, RSR = root to shoot ratio, COT = percentage of cotyledons, Wcot = weight of the cotyledon.

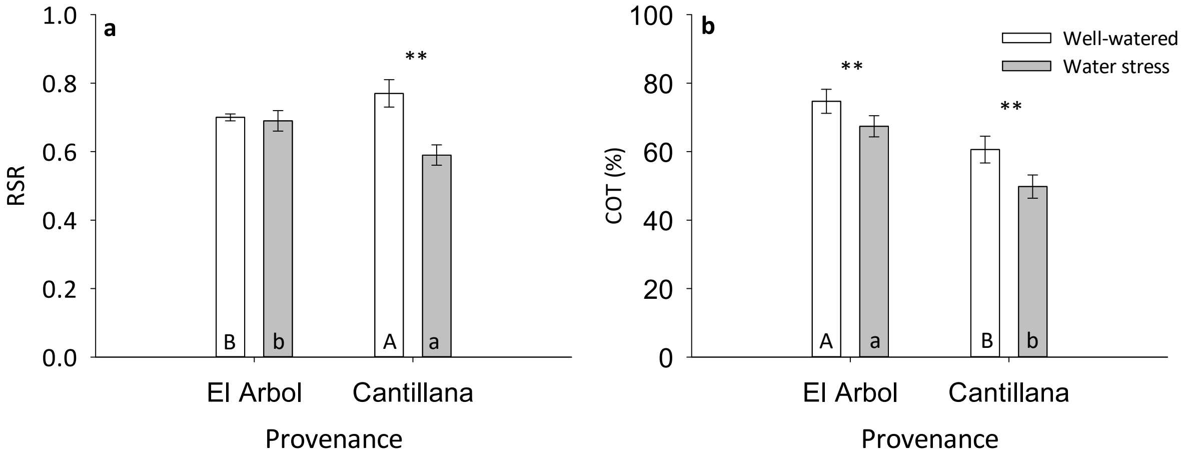

**Fig. 1** Provenance variation by watering regime for RSR (a) and COT (b). ** indicate significant differences within a provenance at P ≤ 0.05. The same lowercase and uppercase letters indicate no significant differences (P ≤ 0.05) between the provenances in the well-watered and water stress treatment.

## Discussion

The provenance effect considerably impacted the germination traits. The coastal and northern provenances El Pobre and Longotoma and the interior plantation Antumapu were not able to survive in large number to the seedling stage, which might be because those provenances are in the dry edge of the species distribution (i.e., the lower the De Martonne aridity index in Table 1), with the consequent low individual performance (Brown 1984). The differences in precipitation, altitude, and temperature where mother trees come from, might have also caused the variations in germination capacity. *B. miersii* is a recalcitrant seeded-species that need moisture to keep its viability. The higher precipitation in the El Arbol than in the other provenances may have partially explain the differences in seed germination, but this needs further research because we used only viable seed in our experiment.

We found that the effect of water was not significant for growth and survival of the provenances but affected RSR and COT. Both parameters are associated with water absorption and carbohydrates reserves within the plant (Bewley and Black 1994; Grossnickle 2005) and may counteract decreases in carbon assimilation during water stress. The provenance El Arbol exhibited an RSR that remained unaffected between watering treatments, suggesting a higher stability in balancing the extraction and losses of water even in a water restricted condition. Seedlings with an adequate size (Villar-Salvador et al. 2012) and a balance between the shoot transpirational surface to the root absorbing surface (i.e., RSR) could have a better chance to survival in sites with water restrictions. Provenance El Arbol also produced larger seedlings, and height is considered a good predictor of seedling survival (Grossnickle and MacDonald 2018), because of the larger roots that allow seedlings to reach moisture in deep soil horizons (Villar-Salvador et al. 2012; Oliet et al. 2009; Cuesta et al. 2010). The higher aboveground growth of the provenance El Arbol could be coupled with a higher water demands, but this is likely compensated by a greater water uptake and food reserves due to its higher mass of cotyledons (Wcot) and roots mass (RDW). The presence of heavier cotyledons is an adaptive character to environmental stresses (Jurado and Westboy 1992) and might explain the high number of seeds that survive to the seedling stage in that provenance.

## Conclusions/ wider implications of findings

Provenances of *B. miersii* from driest locations had low germination capacity and were unable to survive to the seedling stage. On the contrary, the provenance from sites with lower aridity produced larger seedlings with abundant and heavier cotyledons. Differentiation in important functional traits such as the weight of cotyledons suggest that the southern provenances El Arbol could provide appropriate planting material for dry sites; however, the establishment of multi-site and long-term trials are needed to test this hypothesis.

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