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Research note

# Germinación de semillas de *Quercus humboldtii* Bonpl. (Fagaceae): especie vulnerable del bosque Altoandino

# Germination of *Quercus humboldtii* Bonpl. (Fagaceae) seeds: vulnerable species of the high Andean forest

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**Resumen**

El roble colombiano (*Quercus humboldtii*) es una especie endémica, dominante y vulnerable de los bosques altoandinos. Su madera y fruto se han utilizado tradicionalmente en pequeña escala por las poblaciones rurales. Una mayor sincronía del proceso de germinación podría facilitar la producción de plántulas para su uso en los programas de conservación. En vivero, se evaluó la emergencia mediante la inmersión de semillas en cuatro soluciones de ácido giberélico ($GA_3$) por 24 horas (0, 150, 300 y 600 mg $L^{-1}$), a 4 °C. Se adoptó un diseño completamente al azar con cuatro repeticiones de 25 semillas. El tiempo medio de emergencia tuvo una media de 32 días; sin diferencias estadísticamente significativas entre las diferentes dosis. No hubo efecto del $GA_3$ para la emergencia de plántulas hasta 300 mg $L^{-1}$ (media= 92 %); sin embargo, fue desfavorable con 600 mg $L^{-1}$ (20 %). Las semillas frescas de roble andino poseen un alto contenido de humedad (38 %). Lo anterior revela un probable comportamiento recalcitrante. Este estudio confirma, como en la mayoría de los encinos blancos del trópico, la ausencia de latencia y la alta viabilidad de sus semillas. El uso del $GA_3$ no es un tratamiento pregerminativo útil para acelerar y uniformizar la emergencia de plántulas de *Q. humboldtii*.

**Palabras claves:** Bellotas, encino, emergencia de plántulas, latencia, regulador vegetal, roble andino.

**Abstract**

The Colombian oak (*Quercus humdoldtii*) is an endemic dominant and vulnerable species of the high Andean forest. The wood and fruits have traditionally been used on a small scale by rural populations. A greater synchrony of the germination process might facilitate the production of seedlings for conservation plans. In a forest nursery, the emergence was evaluated according to immersion of seeds in four solutions of gibberellic acid ($GA_3$) during 24 h (0, 150, 300 and 600 mg $L^{-1}$), at 4 °C. A completely randomized design was adopted, with four replications of 25 seeds. The mean emergence time was 32 days, without differences between doses. There was no effect of $GA_3$ for the emergence of seedlings up to a dose of 300 mg $L^{-1}$ (mean= 92 %); however, there was a negative effect with 600 mg $L^{-1}$ (20 %). Fresh seeds of the Andean oak have a high moisture content (38 %), perhaps indicative of a recalcitrant behavior. This study confirms, as in most white oaks in the tropic, the absence of dormancy and the high viability of their seeds. The use of $GA_3$ is not a useful pre-germinative treatment for accelerating and synchronizing seedling emergency in this species.

**Key words:** Acorns, oak, seedling emergence, dormancy, growth regulator, Andean oak.

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Within the *Fagaceae* family, the *Quercus* genus includes an extensive group of 450 species known as oaks, which are distributed in the temperate, subtropical and tropical regions (Xia *et al.*, 2012). In Colombia, the Andean or Colombian oak (*Quercus humboldtii* Bonpl.) dominates the three mountain ranges of the country, at an altitudinal range of 700 to 3 500 m (Avella and Rangel, 2014). Their wood and fruits have been used in small scale by the rural populations to elaborate posts, tool handles, crafts, infusions, and coal or firewood as source of domestic energy (Ordóñez *et al*., 2017; Díaz-Rojas e*t al*., 2019).

However, in Colombia, the excessive exploitation of oak populations and the degradation of mountain forests to expand the areas of cattle ranching have generated great pressure, so much that the species is catalogued as in a vulnerable state (Moreno and Cuartas, 2015).

A major constraint to the spread of several oak species is the rapid loss of seed viability, as well as the unsynchronized emergence period, which makes it difficult to select vigorous seedlings in the nursery (Kaliniewicz and Tylek, 2018).

The gibberellins, among them the gibberellic acid ($GA_3$), are plant growth regulators that are used to promote, accelerate and standardize the germination process in forest species (Cabello *et al*., 2019). Although the scarce published information indicates that Andean oak seeds lack latency (Gómez *et al.*, 2013), a better characterization of the emergence and synchronization process would facilitate the production of seedlings for programs for the conservation of the species. The objective of this study was to assess the influence of $GA_3$ on the germination of seeds of *Q. humboldtii*.

The experiment was conducted at the facilities of the University of Pamplona, in Pamplona municipality, *Norte Santander*, Colombia (7°23'23.4" N, 72°38'58.4" W; 2 450 m altitude). According to Holdrige's classification, the region is a low mountain rainforest, with an average temperature of 13.5 °C and 850 mm $year^{-1}$. Specifically, in a greenhouse with a translucent PVC roof and 50 % shade, which was determined with a ceptometer (AccuPAR LP-80, Meter Group), on a sunny day at noon. Temperature and relative humidity were recorded with a datalogger (DT-171, CEM) every 30 min and averaged 15.8 ± 3.6 °C and 75 ± 9.4 %, respectively. The *Q. humboldtii* acorns were purchased from the *Semillas del Bosque* company in *Bogotá*

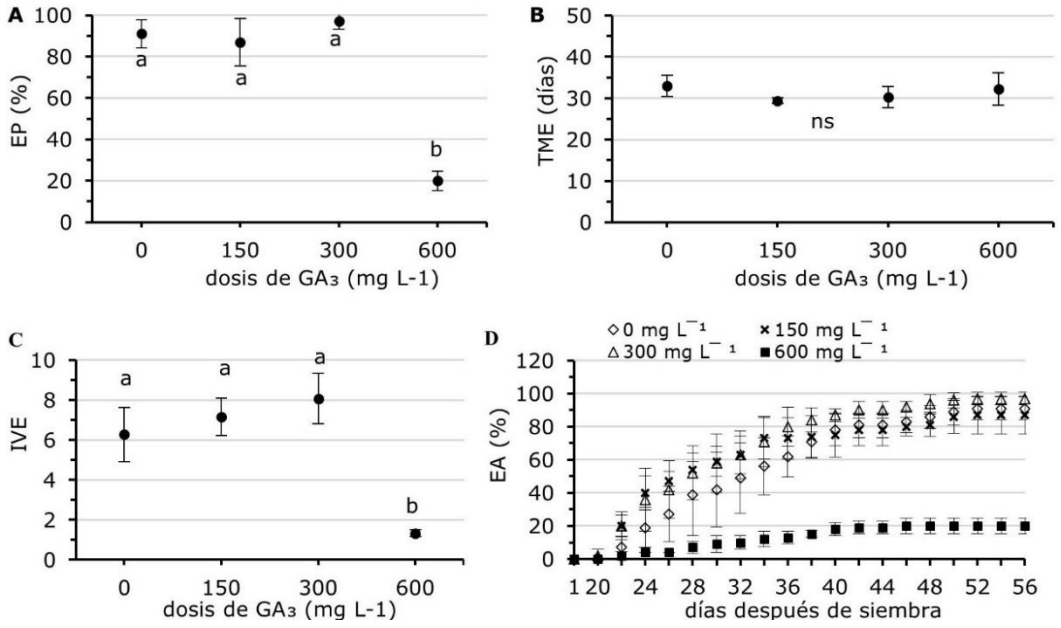
D.C. (Colombia). The seeds were collected in April 2018 and kept in polyethylene bags at 7 °C, until they were received one week later.

Initially, the moisture content of the seeds was determined by averaging three repetitions of 10 seeds held for 17 hours at 103 °C in a (Binder E28) kiln; then the seeds were weighed on an analytical balance (PCB 1000-1, Kern - Sohn) with an accuracy of 0.1 g, and the results were expressed as a percentage. The number of seeds per kilogram and the weight of 1 000 seeds were also calculated, based on the average of five replications of 100 seeds, according to the available quantity (Ministerio da Agricultura, Pecuária e Abastecimento, 2009). 100 seeds were selected at random for length and diameter measurement with a (Ubermann, Rm81) digital caliper (precision 0.01 mm).

The seeds were disinfected with 2 % active sodium hypochlorite during 5 minutes and then washed with distilled water. Sterilized sand (at 75 °C during 72 hours) was used for sowing. This was done (on April 28$^{th}$, 2018) individually in (10 cm wide × 18 cm high) plastic bags, at 1 cm deep, and irrigation was applied when necessary. The experiment was established under a completely randomized design, with four repetitions of 25 seeds per experimental unit, corresponding to the immersion in four different $GA_3$ solutions (0, 150, 300 and 600 mg $L^{-1}$) during 24 hours at 4° C. The treatment without gibberellins consisted in soaking the seeds in distilled water. Those seeds that floated were discarded, because it was presumed that they were not viable, or that they were infested by pests.

About every two days, the number of seedlings that emerged was recorded until the cessation of the process in all treatments. With these data, the following physiological variables were calculated, as proposed by Ranal and Santana (2006): percentage of seedling emergence (SE), mean emergence time (MET), and emergence rate index (ERI) (the Spanish equivalents of these acronyms are *EP*, *TME* and *IVE*, respectively). The assumptions of normality and homoscedasticity of variances were not addressed; therefore, the variables were subjected to non-parametric analysis using the Kruskal-Wallis test ($p<0.05$ value), with the statistical software R 3.3.2 (R Core Team, 2017).

The seeds exhibited a high moisture content (38.3 %), and the weight of 1 000 seeds was 13 279 g, with 75 seeds per kilogram. The average length was 2.9 ± 0.22 cm, and the average width, 2.6 ± 0.3 cm. The application of $GA_3$ at a dose of up to 300 mg $L^{-1}$ did not promote emergence, and there was no statistical difference between the doses of 0 (91 %), 150 (87 %) and 300 mg $L^{-1}$ (97 %); the mean was 92 %. However, at a dose of 600 mg $L^{-1}$ (20 %) the effect was unfavorable (Figure 1A). An equal response was recorded in the ERI, with values of 6.27, 7.17 and 8.08 for doses of 0, 150 and 300 mg $L^{-1}$, respectively (mean= 7.2; Figure 1C). The MET was unaffected by $GA_3$ and exhibited a mean for the 32-day experiment (Figure 1B).




The Spanish equivalents of the acronyms SE, MET, ERI and EA are EP, TME and IVE, respectively.

A = Seedling emergence (SE); B = Mean emergence time (MET); C = Emergence rate index (ERI); D = Accumulated seedling emergence (EA) in seeds of *Quercus humboldtii* Bonpl. treated with $GA_3$. Stockings with the same letter do not differ by Kruskal-Wallis test ($p<0.05$); ns = Not significant. Vertical bars indicate the standard deviation of the mean.

**Figure 1.** Seedling emergence mean times – EP.

The total emergence process was similar between the control treatment, 150 and 300 mg $L^{-1}$; it started at 20 DAP and ended at 52 DAP (Figure 1D). In contrast, seeds treated with 600 mg $L^{-1}$ began to emerge at 24 DAP and reached their maximum value (20 %) at 46 DAP.

In this study, the high moisture content of fresh Andean oak seeds is similar to those indicated for two species of white oaks in Mexico: *Quercus deserticola* Trel and *Quercus rugosa* Nee (50 to 65 %, respectively), whose viability without pre-germination treatments (85 % and 100 %) is also high (Huerta-Paniagua and Rodríguez-Trejo, 2011; Rodríguez-Trejo and Pompa-García, 2016). Most of the seeds of these tropical and subtropical oaks of the *Quercus* subgenus (white oaks) are recalcitrant and have low temperature storage potential, while only a few have intermediate behavior (Xia *et al.*, 2012). *Q. humboldtii*, which belongs to the *Quercus* subgenus, may have this type of seed; however, in order to verify this, it is necessary to evaluate other criteria, such as its tolerance to drying during storage.

The poor synchrony and the long period required to complete the process of emergence of the oak trees may be due to a strategy against natural predators, because their acorns are partially edible and have high contents of protein, lipids and carbohydrates (Liu *et al.*, 2015). This same characteristic has been cited in the Andean oak acorns, whose roasted and ground fruits can be used to prepare a substitute drink for coffee (Díaz-Rojas *et al.*, 2019).

The treatment without $GA_3$ exhibited a high emergence rate; and since, the permeability of the Andean oak seeds was verified, the results suggest that, when they are fresh, they do not present physiological latency (Baskin and Baskin, 2004). Perhaps other pre-germination methods, which have produced an increase in the uniformity and speed of the germination process in oak species, such as cold stratification, scarification, removal of the pericarp or selection of seeds by size and color (Ghasemi and Khosh-Khui, 2007; Huerta-Paniagua and Rodríguez-Trejo, 2011; Liu *et al.*, 2015; Rodríguez-Trejo and Pompa-García, 2016; Kaliniewicz and Tylek, 2018) can be evaluated in *Q. humboldtii* sometime in the future.

This study confirms that the Andean oak has fresh seeds with a high moisture content (38 %) and a high viability (91 %), since there is no evidence of latency. $GA_3$ is not a useful pre-germination treatment for accelerating or standardizing the germination of *Q. humboldtii* seeds.

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**Conflict of interests**

The authors declare no conflict of interests.

**Contribution by author**

Enrique Quevedo: consolidation of the results, and drafting and review of this scientific paper; Andrés Iván Prato: data recording and consolidation, statistical analysis and interpretation of results, drafting and review of this scientific paper.

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