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Phenotypic Variability of *Juglans neotropica* Diels from Different Provenances During Nursery and Plantation Stages in Southern Ecuador

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Abstract

Juglans neotropica Diels, an Andean native species classified as endangered by the IUCN, holds significant potential for reforestation and sustainable forest management programs. This study evaluated seed quality, phenotypic variability, and early establishment under nursery and field conditions in southern Ecuador. Three provenance sites—The Tundo, The Victoria, and The Argelia—were evaluated during the nursery phase, and two (The Tundo and The Victoria) in plantations, applying four pre-germination treatments: control, mechanical scarification, hot water, and water-sun exposure. Parameters assessed included seed weight, size, viability, germination, survival, and growth across three planting environments: secondary forest, riparian forest, and pasture. Significant differences in seed morphometry were observed among localities, while germination was influenced by treatment but not provenance. Seed viability remained high for up to six months, decreasing with a 2% loss of moisture. Survival reached 100% with urea application, and 96% of individuals exhibited straight stems after one year. No significant differences in growth were found between localities; however, basal diameter was highest in the pasture (13.2 mm/year^{-1}), and total height was greatest in the secondary forest (54.8 cm/year^{-1}). These findings provide key technical evidence to optimize the propagation and establishment of *J. neotropica* in ecological restoration and forest production contexts.

Keywords: tropical silviculture; seed quality; phenotypic variability; forest improvement; environmental adaptation

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1. Introduction

The Andean ecosystems in Ecuador, characterized by a delicate balance between ecological fragility and biological vitality, play a crucial role in regional climate regulation, biodiversity conservation, and the sustainable production of valuable resources. Acting as a climatic barrier, the Andes modulate temperature, rainfall, and wind patterns, creating a mosaic of altitudinal zones and microclimates that support a unique and diverse range of species adapted to specific thermal and humidity conditions. These ecosystems sustain essential services such as water regulation, soil fertility, erosion control, and carbon storage, benefitting both local communities and global environmental balance. In addition to

providing medicinal plants and native foods, Andean forests host multipurpose tree species such as *Juglans neotropica* Diels, *Cedrela odorata*, *Ocotea quixos*, and *Polylepis* spp., which offer high-quality timber and deliver significant medicinal, nutritional, and ecological benefits—making them key allies for conservation and sustainable development [1].

J. neotropica is a fast-growing tree that thrives in fertile and humid soils, reaching heights of up to 30 m in natural forests. It is characterized by its thick branches and straight trunk, with an appearance similar to cedar, making it easily distinguishable. With proper silvicultural management, including tree breeding practices, regeneration techniques, soil management, and pest control, this tree can achieve optimal growth and, after 30 to 40 years, provide a sustainable yield of high-quality timber. The implementation of strategies based on ecological and economic principles ensures not only productivity but also the conservation of the forest ecosystem, enhancing its resilience and functionality in the long term. It is considered the most sought-after tree species among the endemic timber flora of the Ecuadorian highlands. It is usually found in small natural groups or isolated among other native trees. Farmers value *J. neotropica* as a long-term source of income due to its fine wood and as a medium-term resource for its edible seeds [2,3].

The sexual germination of the *J. neotropica* species, considered by the International Union for Conservation of Nature (IUCN) as an endangered species, is an essential process not only for commercial purposes but also for the conservation and restoration of global biodiversity. This propagation method enhances genetic variability, which is crucial for adaptability and resistance to diseases, pests, and environmental changes [4]. Sexual reproduction facilitates the restoration of degraded ecosystems by ensuring that plants reintroduced into their natural habitat can adapt and thrive, promoting ecological stability and health [5]. Additionally, the germination of these species holds significant economic and cultural value, as many local communities depend on them for medicinal, food, and material resources [6]. At the ex situ level, seed banks and nurseries play a key role in preserving these species, allowing for their conservation and future reintroduction. Studies highlight the crucial role of these practices in preserving ecosystem health and ensuring the survival of endangered species [2,3].

The establishment of forest plantations with native species from different localities can contribute to genetic improvement and biodiversity conservation when implemented with appropriate ecological criteria and genetic selection programs. Plantations of *J. neotropica* are essential for climate adaptation and ecological restoration, as it improves degraded soils, maintains air and water quality in agroforestry systems, and provides habitat and food for wildlife. This strategy not only promotes genetic variability, which is crucial for species adaptability and resistance to adverse environmental factors such as diseases, pests, and climate changes, but also facilitates the identification and selection of individuals with desirable traits for future conservation and forestry programs [2]. The integration of various localities in a forest plantation allows for the exploration of the species' phenotypic plasticity, evaluating its performance under different edaphoclimatic conditions, which is fundamental for the success of ecological restoration programs and the recovery of degraded ecosystems [5]. Additionally, these plantations are valuable for maintaining essential ecosystem services and offer significant economic and cultural benefits to local communities, who can use these resources to obtain medicinal, food, and material products, thus contributing to their livelihood and well-being [6]. The implementation of forest plantations with native species from different localities is, therefore, a powerful tool for environmental sustainability and socioeconomic development.

In the area relevant to our study, three distinct vegetation strata associated with *J. neotropica* are identified: (i) secondary forest, characterized by natural regeneration following both anthropogenic and natural disturbances and marked by high biodiversity

and active ecological succession; (ii) riparian forest, located along the ravine of the Zañe micro-watershed, shaped by specific vegetative successions linked to natural regeneration processes; and (iii) pasture stratum, composed of grassland used for livestock activities, with scattered trees and ongoing vegetative recovery. Each stratum presents unique features in terms of biodiversity, succession dynamics, and land use, making them essential for understanding ecosystem processes and informing sustainable management strategies [1–3].

The purpose of the research was to optimize the propagation and establishment of *J. neotropica* in ecological restoration and forest production by evaluating seed quality, phenotypic variability, and early establishment in nursery and field conditions—key aspects for the species' adaptability and resistance to diseases, pests, and climate changes [4]. This approach allows for the evaluation of the species' phenotypic plasticity in a common environment, facilitating the selection of superior genotypes that can thrive under adverse environmental conditions [5]. Additionally, this practice contributes to ex situ conservation, providing a genetic reserve for future reintroductions and scientific studies [6]. The integration of various localities also helps identify the best management and conservation practices, ensuring the long-term sustainability of the species and its associated ecosystems.

Despite the ecological and economic relevance of *J. neotropica* in the Andean region, there is limited knowledge regarding the influence of seed provenance, pre-germination treatments, and environmental conditions on its germination and early growth. This study aims to address this gap by evaluating seed quality, phenotypic variability, and initial seedling performance in nursery and field conditions. The objective is to optimize propagation and establishment strategies that enhance genetic diversity, promote resilience to biotic and abiotic stressors, and support both conservation and sustainable forest production efforts.

2. Materials and Methods

2.1. Study Area

The study area is located on the private estate Hacienda "The Florencia," which is situated within one of the micro-watersheds of The Zañe hill, in the Carigan parish of Loja canton and province. It has a total area of 115.8 hectares, with an altitudinal range from 2024 to 2800 m above sea level [7].

Of the 115.8 hectares of the private Hacienda "The Florencia", 93 hectares are located within the Zañe micro-basin, which comprises two distinct ecosystems. The first ecosystem is a Montane Evergreen Forest in the southern region of the Eastern Cordillera of the Andes, characterized by high fragility, medium-level threats, and high vulnerability, with moderate fragmentation and connectivity [7]. It spans an altitudinal range of 2200–3000 m above sea level, with a minimum temperature of 5.4 °C and a maximum of 17.6 °C, receiving an average annual precipitation of 2894 mm.

The second ecosystem is a Lower Montane Evergreen Forest in the southern region of the Eastern Cordillera of the Andes, situated within an altitudinal range of 1660–2200 m above sea level. It has a tropical climate, with an average temperature of 12.9 °C, a maximum of 25.4 °C, and an average annual precipitation of 2245 mm. These ecosystems are located on slopes with moderate inclines and rocky soils and are recognized for their high biodiversity, hosting a wide variety of plant species, birds, mammals, and other taxonomic groups [7].

Lastly, the micro-basin that contains the private Hacienda "The Florencia" has an approximate area of 148 hectares, within an elevation range of 2015 to 2815 m above sea level.

2.2. Study Description

The research was conducted in two phases. In the first phase, seeds from *Juglans neotropica* Diels trees were germinated, selected based on desirable phenotypic traits proposed by [8], and sourced from three specific localities: The Victoria, The Tundo, and The Argelia [7]. The second phase involved the establishment of a plantation trial with *J. neotropica* from two distinct localities, The Victoria and The Tundo, within the grounds of Hacienda The Florencia (Figure 1).

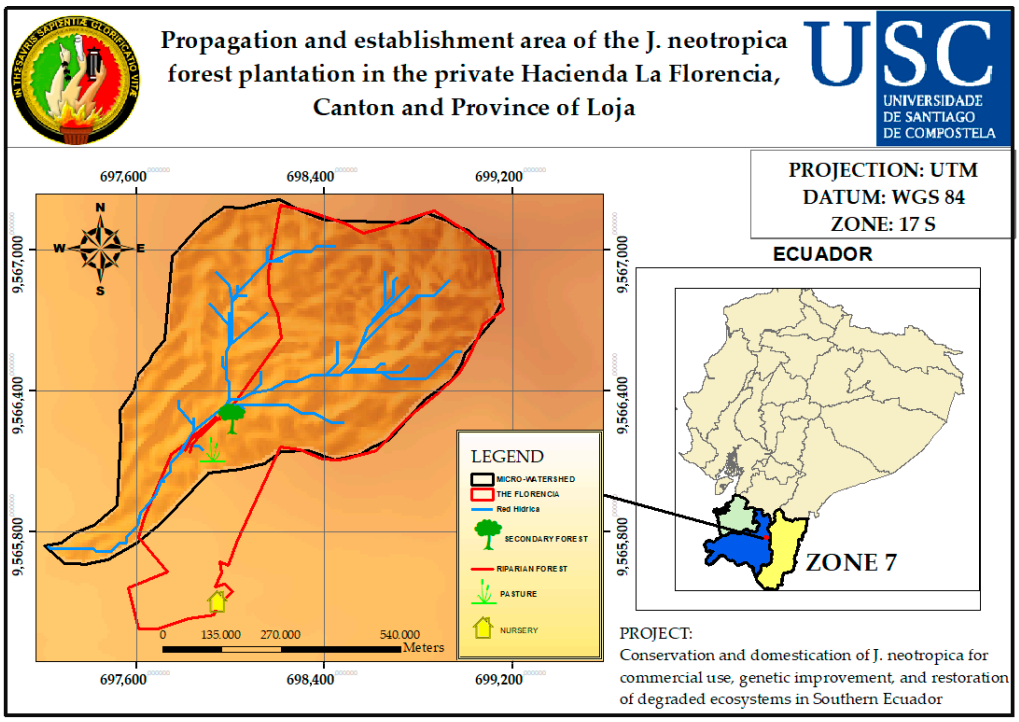


Figure 1. Locality map of the propagation and establishment of *Juglans neotropica* Diels in three stages of plant succession: secondary forest, riparian forest, and pasture.

2.3. First Phase: At the Nursery Level

To comply with the nursery phase of this research, the methodology proposed by [9] was followed. During this stage, the primary objective was to propagate, observe, and monitor the vegetative development of seedlings from three different localities before transferring them to the field or final planting site. To achieve this, various pre-germination treatments were implemented to assess their impact on germination percentage, growth, and development.

2.3.1. Quality Parameters of *J. neotropica* Seeds

A representative sample of the batch was selected for the proposed methodology in the quality analysis of *J. neotropica* seeds, strictly following the procedures established by the International Seed Testing Association (ISTA) [10].

1. Purity Percentage: To evaluate seed purity, a comprehensive physical analysis was conducted. First, a physical test was performed, involving the meticulous separation of seeds from possible impurities such as sand, soil, stones, plant debris, and other waste. This process aimed to ensure sample cleanliness and quality by removing any undesired elements. Subsequently, the genetic purification stage was carried out, with the objective of separating the analyzed seeds from other crops or weeds present. This phase is crucial to ensure sample homogeneity and obtain precise results regarding seed purity. Purity was quantified as the percentage by weight of pure

seeds in relation to the total sample analyzed. This comprehensive methodology guaranteed an exhaustive evaluation of seed purity, which is essential for quality and performance in agroforestry projects. To determine seed purity, five seed lots of one kilogram each were randomly selected, containing impurities. The following formula was applied for the assessment (Figure 2).

2. The number of seeds per kilogram was determined by randomly selecting seeds until reaching one kilogram. This process was repeated five times, and an average number of seeds per kilogram was subsequently calculated (Figure 3). To ensure consistency across all samples, seeds were weighed using a digital precision scale (Camry Electronic Scale Model EK5350, Zhongshan, Guangdong, China).
3. Moisture Content Percentage (% MC): For the determination of moisture content in *J. neotropica* seeds, 25 seeds were selected as samples for each locality. Subsequently, the initial weight of each seed was recorded before subjecting them to a storage process. Since these are recalcitrant seeds, they have a hard appearance but are delicate and lose their viability quickly after being extracted from the fruit. This type of seed typically requires specific conditions and special care for storage and preservation. For this reason, they were stored for a period of six months at an ambient temperature of 18 °C. Gradual weighing was carried out at the beginning (time 0) and six months after collection to determine the percentage of moisture lost during the established period (Figure 4).



Figure 2. *J. neotropica* seeds with impurities (**a**) and without impurities (**b**).



Figure 3. Average number of *J. neotropica* seeds per kilogram.



Figure 4. Moisture content of *J. neotropica* seeds: (**a**) 0 months; (**b**) 6 months.

The moisture content was calculated using the following equation:

$$MC = \left(\frac{Imw - Fmw}{Imw} \right) * 100 \quad (1)$$

where the variables are as follows:

MC = Moisture content (%);
Imw = Initial moisture weight of the samples (g);
Fmw = Final moisture weight of the samples (g).

4. Germination Percentage: The methodology for determining the germination percentage, following the standards of the International Seed Testing Association, adhered to a standardized procedure that ensured both accuracy and reproducibility of results. For this study, a representative sample of seeds was selected based on criteria of uniformity in size, maturity, and physiological state. These seeds were stored under controlled conditions, maintaining an average temperature of 18 °C and a relative humidity of 70%, parameters defined from previous studies and adjusted to the physiological requirements of *J. neotropica* in its natural habitat. During the storage period, systematic monitoring of viability was conducted, recording variations in moisture and physiological state to minimize the influence of external environmental factors. These conditions allowed for the collection of precise data on conservation and contributed to promoting germination. Subsequently, the germinated seeds were evaluated and classified according to defined criteria, such as normal or abnormal development. The germination percentage was calculated by dividing the number of normal seedlings by the total number of seeds sown and multiplying the result by 100, thus providing a reliable indicator of seed quality. This internationally recognized and adopted method ensured uniformity in assessing seed viability on a global scale.

In this study, four pre-germination treatments were conducted to evaluate the effectiveness of each method:

a. **Mechanical treatment:** The seeds were placed on a solid surface and struck with a hammer until they cracked.
b. **Hot water imbibition:** The seeds were placed in a container with hot water at 100 °C, at a ratio of 4 to 5 times their volume, and removed after two minutes of immersion in the heat source.
c. **Cold water imbibition and sun exposure:** To remove inhibitors, the seeds were exposed to sunlight during the day and soaked in water at night. This process was repeated for three consecutive days and nights.
d. **Control treatment:** This method served as a baseline for comparing the effectiveness of the other treatments.

The number of germinated seeds was recorded daily, and the germination percentage was calculated. This process was repeated with different seed samples corresponding to each repetition of the applied experimental treatment, ensuring that each represented a specific condition of the study. The treatments were conducted under natural environmental conditions, allowing for the collection of representative and reliable data on germination performance in response to the evaluated variables.

2.3.2. Phenotypic Characteristics of *J. neotropica*

a. **Seed size and weight:** To determine the size of *J. neotropica* seeds, a detailed methodology was implemented. Seed samples from different progenies were collected from various geographical localities. Subsequently, precise measurements of each seed's size were taken using a digital caliper (Mitutoyo, Kawasaki, Japan). This process was systematically repeated for each locality, where seed samples consisting of 100 units were evaluated. These experimental samples, referred to as seed lots, allowed for the quantification of inherent variability within each sampled population. The applied methodology ensured rigorous statistical representation, facilitating the generation of consistent and reproducible data on germination performance under diverse environmental conditions (Figure 5).



Figure 5. Measurement of the diameter (**a**) and measurement of the height (**b**) of the *J. neotropica* seed.

b. **Seedling height at the nursery level (Th, cm):** This morphological parameter is related to the plant's photosynthetic capacity and transpiration surface. It corresponds to the length from the root collar to the apex of the main stem, measured in cm. It was measured for all living plants in the experimental unit six months after germination for all localities.

c. **Basal diameter of seedlings (Bd, mm):** The basal diameter is a key indicator of the plant's ability to transport water to the aerial parts, as well as its structural resistance and relative tolerance to high-temperature conditions. This parameter was measured at the height of the root collar, at the transition point between the root system and the base of the stem, in all living plants of the experimental unit. Measurements were conducted six months after germination, covering all the localities included in the study.

d. **Number of leaves per treatment:** This morphological parameter was evaluated in all living individuals of the experimental unit propagated in the nursery under the different treatments. Measurements were conducted six months after germination, coinciding with the scheduled evaluations of the other morphological variables and covering all localities included in the study. In each case, the number of leaves was recorded using the same assessment framework, allowing for comparative analysis of foliar development among the different origin localities [11,12] (Figure 6).



Figure 6. Measurement of dasometric variables at the nursery level, such as (**a**) total height (Th, cm) and (**b**) basal diameter (Bd, cm) of *J. neotropica* from different localities.

2.3.3. Experimental Design at Nursery Level

A completely randomized block design (CRBD) with a bifactorial arrangement and three replications was used (Figure 7).

- **Functional analysis:** The coefficient of variation was calculated to assess data dispersion. Additionally, mean separation was analyzed using Tukey's test at a 5% significance level to identify statistical differences among treatments and localities.
- **Factors under study:**

  Factor 1 (pre-germination treatments):
  T0 = Control (seeds without any treatment);
  T1 = Immersion in boiling water at 100 °C (2 min);
  T2 = Mechanical (cracked with a hammer);
  T3 = Immersion in water and exposure to sunlight (three days in ambient water and three days in the sun).
  Factor 2 (localities):
  L1 = The Tundo;
  L2 = The Victoria;
  L3 = The Argelia.
- **Treatments under study:** The combination of the factors under study resulted in 12 treatments, which are detailed below (Table 1).
- **Experimental field specifications:**

  Number of treatments: 12;
  Number of replications: 3;
  Total number of units: 36;
  Total trial area: 40 m^2;
  Total number of seeds per plot: 100;
  Total number of seeds per treatment: 25.

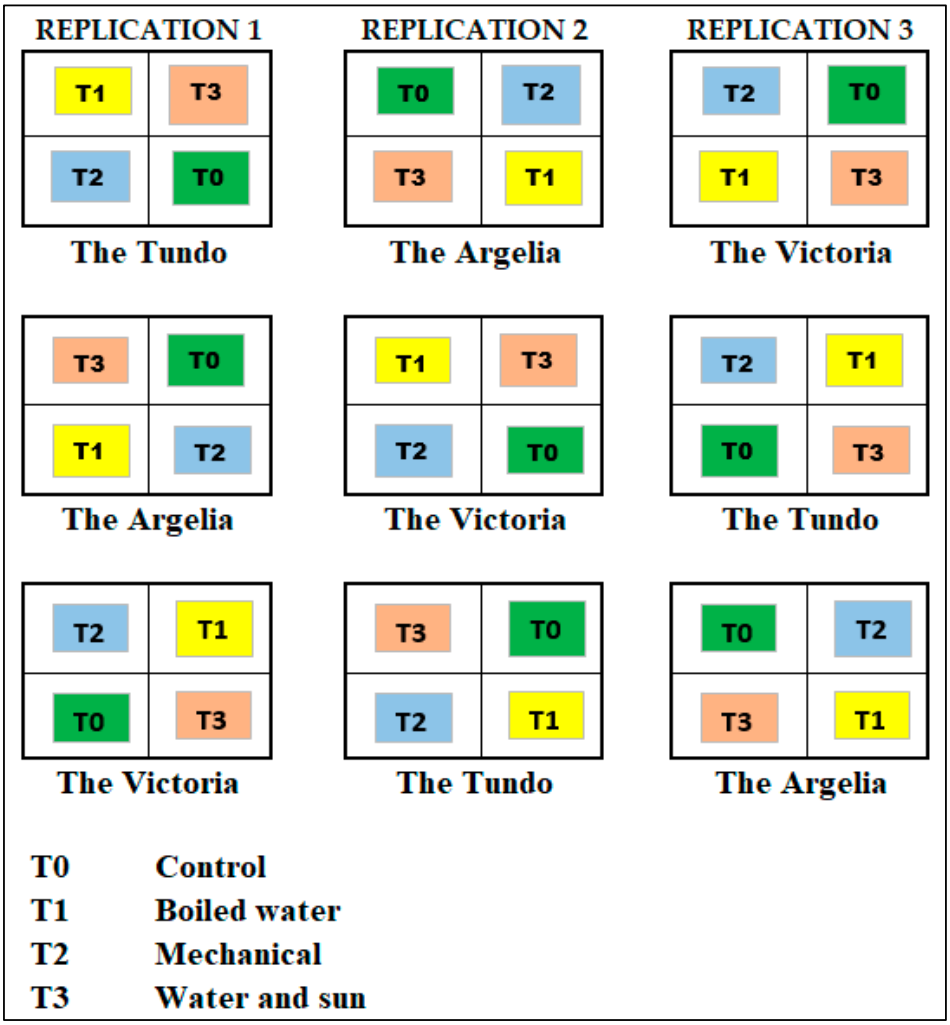


Figure 7. Experimental design of pre-germination treatments by localities.

- **Dependent variables used in the statistical analysis:**

In this study, the main dependent variable in the germination analysis was the germination percentage, which was measured for each treatment and locality. Additionally, other seed traits such as weight, height, and diameter were evaluated using the same experimental design, and separate statistical analyses were conducted to identify significant differences among treatments and origin localities.

- **Mathematical model for the statistical design:**

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \epsilon_{ijk} \quad (2)$$

Y_{ijk} : the dependent variable;
μ: the global mean of all treatments;
α_i : (alpha) factor 1 under study;
i: the levels of factor;
β_j : (beta) factor 2 under study;
j : the levels of factor 2;
$(\alpha\beta)_{ij}$: the interaction between the i-th level of the pre-germination treatment and the j-th level of the locality of origin;
γ_k : random effect of replication;
ϵ_{ijk} : the experimental error of the ijk observation.

Table 1. Germination treatments of *Juglans neotropica* Diels seeds evaluated from three study areas in the nursery.

N° Treatments	Code	Description
1	T0L1	Control—The Tundo
2	T0L2	Control—The Victoria
3	T0L3	Control—The Argelia
4	T1L1	Boiling water—The Tundo
5	T1L2	Boiling water—The Victoria
6	T1L3	Boiling water—The Argelia
7	T2L1	Mechanical—The Tundo
8	T2L2	Mechanical—The Victoria
9	T2L3	Mechanical—The Argelia
10	T3L1	Water and sun—The Tundo
11	T3L2	Water and sun—The Victoria
12	T3L3	Water and sun—The Argelia

In addition to germination percentage, other morphological and seed quality variables were evaluated during Phase I, including seed size and weight, moisture content, purity, and number of seeds per kilogram, as well as seedling height, basal diameter, and number of leaves. These variables were measured six months after sowing, using the same experimental design and statistical methods described for germination analysis.

2.4. Second Phase: At the Level of Forest Plantation

In the second phase, a plantation trial of *J. neotropica* was established using germinated plants from two distinct localities of origin (The Tundo and The Victoria), under uniform planting conditions. These belong to a Piedmont Semi-deciduous Forest ecosystem of Catamayo-Alamor, differing from a Lower Montane Evergreen Forest ecosystem in the southern part of the eastern Andes mountain range, with an altitudinal range of 1660–2200 m.a.s.l. This activity was carried out following [13] methodology, which consisted of applying the following steps:

2.4.1. Selection of the Area to Be Planted

The selection of the planting site was based on the edaphoclimatic suitability for *J. neotropica*, prioritizing areas historically occupied by the species but currently degraded due to deforestation, land-use changes, landslides, and livestock grazing. To support ecological restoration efforts, three types of environments—referred to as strata in the study—were selected: secondary forest, riparian forest, and pasture. Prior to planting, detailed soil analyses were conducted to assess their physicochemical properties.

In the secondary and riparian forest sites, the soil exhibited moderately acidic conditions (pH = 5.32), high nitrogen content (51 ppm), and medium levels of phosphorus (19 ppm), potassium (0.3 meq/100 mL), calcium (5 meq/100 mL), and magnesium (0.9 meq/100 mL). These results indicate moderate fertility, with potential phosphorus limitations and a soil reaction that may require liming to optimize nutrient uptake.

In contrast, the pasture sector showed a slightly less acidic pH (5.63), greater nitrogen availability (54 ppm), medium phosphorus levels (14 ppm), and an adequate concentration of exchangeable bases, with a notably high magnesium value (1.5 meq/100 mL).

Climatic conditions across both sites were relatively homogeneous, which favored the standardization of the planting trial.

Additional selection criteria included local biodiversity, ecological threats, and interspecific competition, all of which were considered in the restoration design.

2.4.2. Selection and Field Establishment of Plants

Selected *J. neotropica* plants propagated in the nursery were chosen for transplanting based on vigor, root development, stem lignification, foliar health, and absence of pests or diseases. For their establishment, a square planting layout (5 × 5 m) was manually implemented over a 2500 m^2 area per stratum. Standard silvicultural practices were followed for plot delineation, seedling positioning, and hole excavation (30 × 30 × 30 cm). Seedlings were carefully transplanted to ensure optimal root positioning and soil contact, which facilitated uniform establishment and subsequent monitoring across the plantation trials.

2.4.3. Fertilization

Finally, based on the results of prior soil analyses conducted in the three selected strata, a fertilization scheme was designed to evaluate the performance of *J. neotropica* under different levels of nutrient availability. The analyses revealed soils with moderately acidic pH, medium fertility, and potential phosphorus deficiencies, which justified the implementation of differentiated nutritional treatments. Three fertilization levels were established using combinations of NPK (13-40-13) and urea, corresponding to low, medium, and high doses, applied in liquid form directly into each planting hole. This method aimed to optimize nutrient uptake by the seedlings and reduce leaching losses, considering the high rainfall in the study area. The methodological objective was to identify the most efficient fertilization regime for the early establishment of the species under ecological restoration conditions.

2.4.4. Management

The management of a forest plantation during the first year included essential activities such as regular irrigation to ensure proper root establishment and initial growth, weed control to prevent competition for resources, constant protection against pests and diseases through monitoring and appropriate treatments, fertilization to provide necessary nutrients, replacement of non-surviving seedlings to maintain the planned density, and continuous supervision of the plantation's condition to adjust management practices as needed, thus ensuring the successful establishment and healthy development of the seedlings.

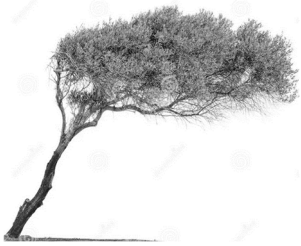
Subsequently, adaptability parameters were evaluated, such as survival rate and stem straightness of the plants in the final site, as well as phenotypic characteristics of dendrometric variables such as total height and basal diameter of the plants at the first year of planting, following the methodology proposed by [13].

2.4.5. Plant Response to Plantation Conditions

a. **Survival rate (%):** The survival rate (%) was determined based on an evaluation conducted 60 days after the trial establishment. To achieve this, the number of established plants in each experimental unit was quantified and calculated as the ratio of surviving individuals to the total number of planted trees. This calculation was performed using the following equation: (number of established plants/total number of planted trees) × 100. The evaluation was carried out under standardized conditions, ensuring uniformity in the application of establishment criteria, which allows for the interpretation of the plants' adaptability to the planting site and their response to the environmental conditions of the study area.

b. **Stem straightness at 12 months:** To carry out the phenotypic development assessment, a methodology based on shape codes was implemented, following the guidelines proposed by [14,15]. The individuals under study were evaluated at twelve months to determine their physical characteristics. These categories provided a comprehensive framework for quantitatively describing stem shape, allowing for a more precise evaluation of phenotypic characteristics across four categories (Table 2).

Table 2. Classification of stem morphology into four categories.

Category	Interpretation	Illustration
Sinuous	Twisted, tortuous, or crooked, with curved and serpentine shapes	
Inclined	Indicates an inclination or slope	
Bifurcated	Divided into two branches or parts	
Straight	A line or surface without curves or angles	

2.4.6. Phenotypic Traits Based on Dendrometric Variables

1. Basal diameter (BD, cm)

The stem diameter (cm) was measured at the base of 12-month-old *J. neotropica* plants using a caliper.

2. Total height (m)

The total height of the plant (m) was measured with a graduated ruler, from the base at ground level to the apex, at 12 months after establishment in the definitive site.

2.4.7. Experimental Design at Plantation Level

In the study area, three stages of natural succession were selected, which in this research were identified as strata (secondary forest, riparian forest, and pasture). The purpose was to group units with similar conditions into strata so that the sampling units within each stratum were as homogeneous as possible, while the strata themselves remained heterogeneous [16].

A completely randomized block design (CRBD) was used in each stratum with a factorial arrangement, with five replications, considering each plant as the sampling unit (Figure 8).

1. **Functional analysis:** The coefficient of variation was determined, and the mean separation was analyzed using Tukey's test at a 5% error level with a 95% confidence interval.
2. **Factors under study:** Two study factors were analyzed for each of the three strata (secondary forest, riparian forest, and pasture), as detailed below.

   Factor A: Localities

   L1 = The Tundo;
   L2 = The Victoria.

   Factor B: Fertilizers

   F0 = Control;
   F1 = Urea;
   F2 = NPK.
3. **Treatments under study:** The combination of the factors under study resulted in 8 treatments, which are detailed below (Table 3).
4. **Experimental unit:** A 50 × 50 m (2500 m^2) plot was used in each stratum, with four rectangular subplots of 25 × 25 m (625 m^2). Two subplots corresponded to the forest site of The Tundo and the other two to The Victoria. Each subplot contained five experimental units measuring 5 × 25 m (125 m^2), with each unit including five plants, where each plant was considered a replicate. This resulted in a subtotal of 10 experimental units per forest site and a total of 20 experimental units across both sites.
5. **Localities:** Two localities were evaluated, one from the Sozoranga canton and the other from the Macará canton, both belonging to the Loja province in southern Ecuador.

   L1 = The Tundo
   L2 = The Victoria
6. **Mathematical model for the statistical design:** To statistically analyze and determine whether there are significant differences among the treatments tested in *J. neotropica*, an ANOVA (analysis of variance) was applied.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \epsilon_{ijk} \quad (3)$$

Y_{ijk} : the dependent variable;
μ: the global mean of all treatments;
α_i : (alpha) factor 1 under study;
i: the levels of factor;
β_j : (beta) factor 2 under study;
j : the levels of factor 2;
$(\alpha\beta)_{ij}$: the interaction between the i-th level of the locality of origin and the j-th level of the fertilizer;

γ_k : random effect of replication;

ϵ_{ijk} : the experimental error of the *ijk* observation.

7. **Data analysis**

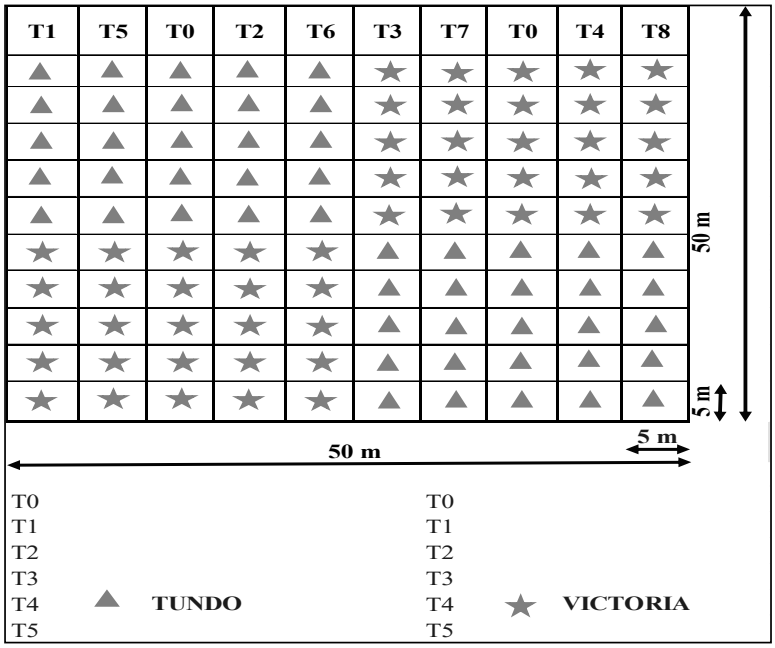


Figure 8. Completely randomized block experimental design in the lands of The Hacienda "The Florencia" to evaluate the behavior of two provenances of *J. neotropica* plants.

Table 3. Treatments evaluated in the present research at the plantation level in the strata: secondary forest, riparian forest, and pasture.

FACTORS		Levels	Treatment
F1: Location	**F2: Fertilizer**		
L1: The Tundo	Fert 0:	**Control**	T0
	Fert 1: NPK (13-40-13)	**NPK 1:** 100 gr	T1
		NPK 2: 150 gr	T2
		NPK 3: 200 gr	T3
		NPK 4: 250 gr	T4
	Fert 2: Urea	**Urea:** 100 gr	T5
		Urea: 150 gr	T6
		Urea: 200 gr	T7
		Urea: 250 gr	T8
L2: The Victoria	Fert 0: 0	**Control**	T0
	Fert 1: NPK (13-40-13)	**NPK 1:** 100 gr	T1
		NPK 2: 150 gr	T2
		NPK 3: 200 gr	T3
		NPK 4: 250 gr	T4
	Fert 2: Urea	**Urea:** 100 gr	T5
		Urea: 150 gr	T6
		Urea: 200 gr	T7
		Urea: 250 gr	T8

To evaluate significant differences ($p < 0.05$) among the different treatments, a mean comparison was performed using Tukey's test with InfoStat Professional 2020 [17].

The statistical analysis was conducted separately for each of the three identified strata (secondary forest, riparian forest, and pasture) to maintain internal homogeneity of the sampling units within each stratum. Consequently, the stratum was not included as a factor in the model but was treated as an independent analytical structure, with a completely randomized block design and factorial arrangement applied to each one.

3. Results

3.1. Phase 1: At the Nursery Level

3.1.1. Quality Parameters of *Juglans neotropica* Diels Seeds

1. Purity percentage

The physical purity of *J. neotropica* seeds showed a high proportion of pure seeds relative to total sample weight, indicating good seed lot quality. The results revealed clear differences in the composition of seed components, as shown in Table 4.

Table 4. Mean ± standard deviation of seed impurity and number of seeds per kilogram of *J. neotropica* by provenance.

Locality	Impurity (%) ± SD	Seeds kg^{-1} ± SD	CV Seeds (%)
The Tundo	1.08 ± 0.22	30.0 ± 1.87	6.23
The Victoria	0.88 ± 0.22	32.0 ± 2.24	7.00
The Argelia	1.04 ± 0.29	35.0 ± 1.87	5.34

Legend: Summary of physical purity and seed count per kilogram for *J. neotropica* seeds collected from three localities. Values represent the mean ± standard deviation based on five replicates.

2. Number of seeds per kilogram (kg)

The results revealed significant variations in seed density per kilogram among the different localities of *J. neotropica*, with a value of 30 seeds per kilogram in The Tundo, which had the largest seed sizes, followed by The Victoria with 32 seeds kg^{-1}, and finally The Argelia with 35 seeds kg^{-1} (Table 4).

3. Percentage of moisture content (MC %)

The results clearly show that seeds from the three localities gradually lose moisture over time in months, and their 100% viability has been confirmed up to six months of storage. Beyond that period, it has been demonstrated that even a 2% moisture loss leads to a loss of viability (Table 5).

4. Germination percentage

The analysis of variance (ANOVA) revealed a significant interaction between pre-germination treatment and locality of origin ($p \leq 0.05$). This interaction was mainly evident in the control treatment (T0), where The Victoria exhibited a significantly higher germination percentage compared to The Tundo and The Argelia, indicating a differential response in the absence of pre-germination treatments.

In contrast, treatments T1, T2, and T3 showed no significant differences among localities, as confirmed by the post hoc analysis, suggesting that the pre-germination treatments produced a more uniform germination response across environments (Figure 9).

Table 5. Changes in the moisture content and viability of *J. neotropica* seeds over time by locality. Mean values ± SD.

Sampling Time	Locality	N° Seeds	Wws (g)	Dws (g)	Mc (%)	Viability
Measurement 1 (starting month)	The Tundo	25	31.3 ± 0.2	31.1 ± 0.2	0.64 ± 0.03	Viable
	The Victoria	25	28.7 ± 0.3	28.5 ± 0.3	0.73 ± 0.04	Viable
	The Argelia	25	25.6 ± 0.2	25.4 ± 0.2	0.51 ± 0.02	Viable
Measurement 2 (3 months)	The Tundo	25	31.3 ± 0.2	30.9 ± 0.2	1.34 ± 0.05	Viable
	The Victoria	25	28.7 ± 0.3	28.3 ± 0.3	1.46 ± 0.06	Viable
	The Argelia	25	25.6 ± 0.2	25.2 ± 0.2	1.37 ± 0.05	Viable
Measurement 3 (6 months)	The Tundo	25	31.3 ± 0.2	30.6 ± 0.3	2.17 ± 0.07	Not viable
	The Victoria	25	28.7 ± 0.3	28.0 ± 0.3	2.37 ± 0.08	Not viable
	The Argelia	25	25.6 ± 0.2	24.9 ± 0.2	2.58 ± 0.09	Not viable

Wws: fresh weight; Dws: dry weight; Mc: moisture content.

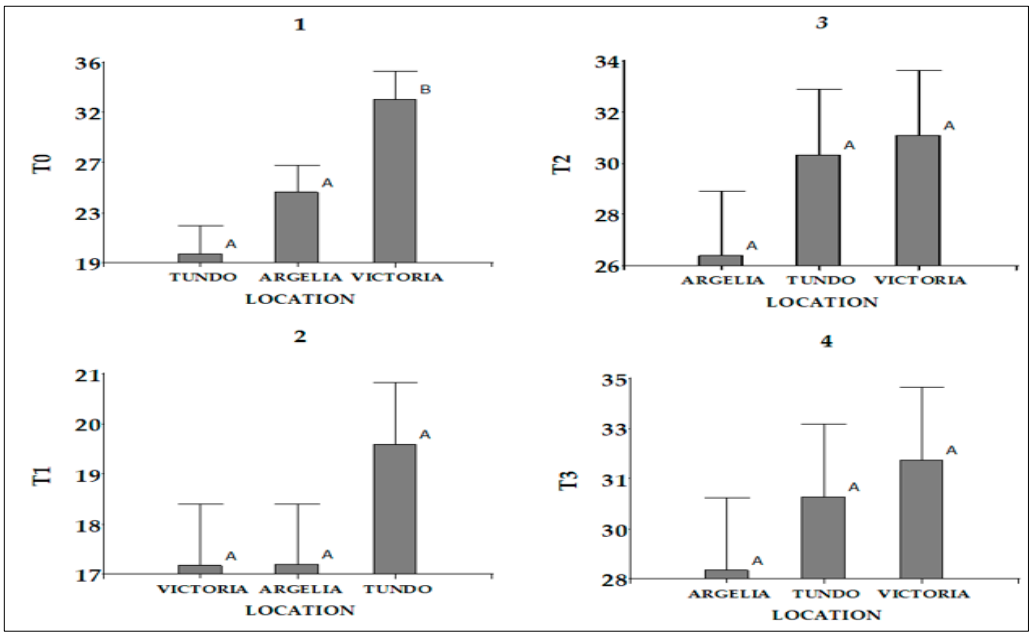


Figure 9. Analysis of variance (ANOVA) of germination percentage for each pre-germination treatment across all localities. A significant interaction between treatment and locality was detected ($p \leq 0.05$). Different letters indicate statistically significant differences among localities within each treatment. Numbers (**1–4**) correspond to the pre-germination treatments: 1 = T0, 2 = T1, 3 = T2, 4 = T3.

3.1.2. Germination Percentage Among Treatments and Localities

Statistically significant differences were observed among localities at each time point (T0–T3), as indicated by non-overlapping letters in the post hoc analysis. At T0, The Victoria exhibited the highest value, significantly greater than The Argelia and The Tundo. At T1, although values were closer, The Tundo remained significantly higher than the other two localities. During T2 and T3, all three localities differed significantly, with The Victoria maintaining the highest mean values (Figure 10).

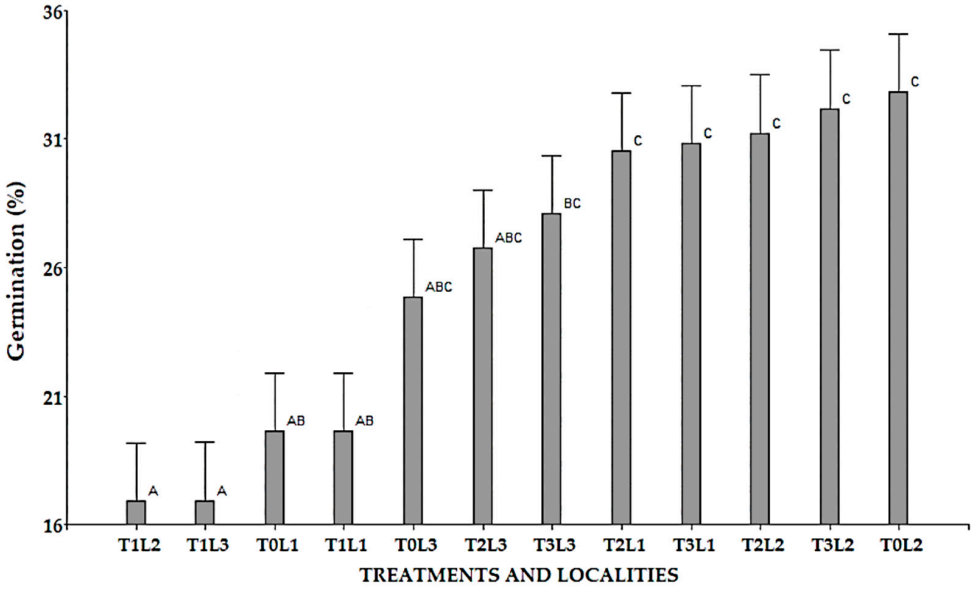


Figure 10. Analysis of variance of the average germination percentage across treatments and localities. Different letters indicate significant differences ($p \leq 0.05$).

5. Phenotypic Characteristics of *J. neotropica* Seeds

3.1.3. Size and Weight of the Seeds

Clear phenotypic differences were observed in the size and weight of *J. neotropica* seeds depending on their locality of origin. Seeds from The Tundo exhibited the highest average values, with a weight of 29.55 ± 6.15 g, a diameter of 40.70 ± 3.30 mm, and a height of 39.75 ± 3.68 mm. These were followed by seeds from The Victoria, with an average weight of 24.39 ± 4.67 g, a diameter of 36.88 ± 3.01 mm, and a height of 37.40 ± 3.23 mm. The smallest seeds were recorded from the locality of The Argelia, with average values of 20.93 ± 6.06 g in weight, 35.99 ± 3.88 mm in diameter, and 32.81 ± 4.47 mm in height. This variability suggests possible differences in the quality of genetic material or in local environmental conditions that may be influencing seed development (Table 6).

Table 6. Descriptive statistics of seed weight, diameter, and height of *J. neotropica* collected from three localities.

Locality	Variable	*n*	Mean ± SD	Min–Max
The Argelia	Weight (g)	331	20.93 ± 6.06	(8.00–40.00)
	Diameter (mm)	331	35.99 ± 3.88	(25.70–46.70)
	Height (mm)	331	32.81 ± 4.47	(24.90–79.10)
The Tundo	Weight (g)	333	29.55 ± 6.15	(14.00–46.00)
	Diameter (mm)	333	40.70 ± 3.30	(29.80–48.50)
	Height (mm)	333	39.75 ± 3.68	(25.30–49.20)
The Victoria	Weight (g)	101	24.39 ± 4.67	(13.00–38.00)
	Diameter (mm)	101	36.88 ± 3.01	(30.40–58.20)
	Height (mm)	101	37.40 ± 3.23	(27.20–45.20)

Values are expressed as mean ± standard deviation, with minimum and maximum in parentheses.

The summarized results of seed size and weight for each locality are shown in Table 6 and Figure 11, where significant differences can be observed.

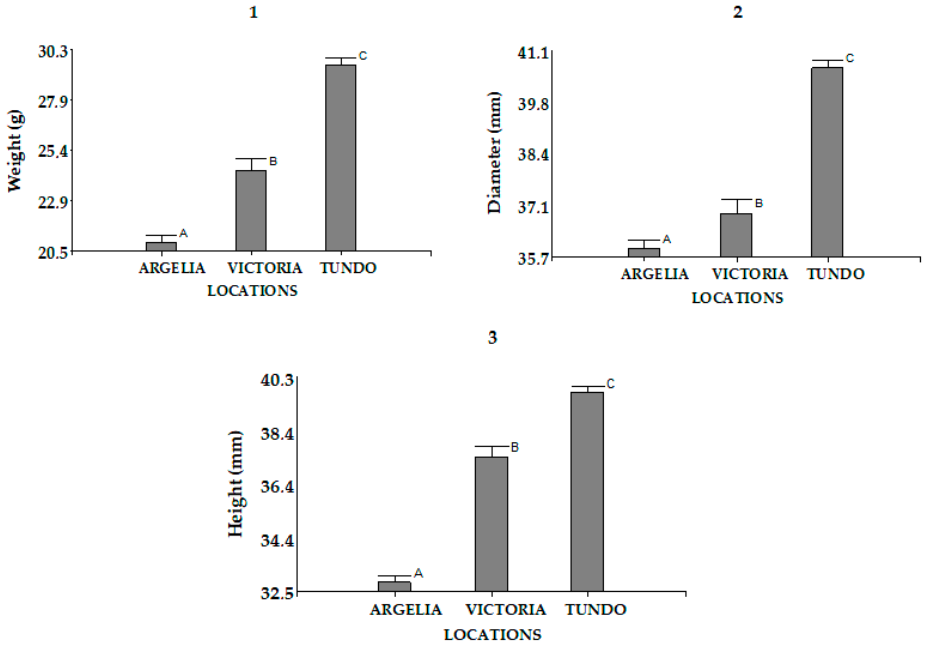


Figure 11. Analysis of variance of (**1**) weight (g), (**2**) diameter (mm), and (**3**) height (mm) of *J. neotropica* seeds across different localities. Different letters (A, B, C) indicate statistically significant differences among the evaluated localities ($p \leq 0.05$).

3.1.4. Basal Diameter, Total Height, and Number of Leaves of the Nursery Plants

Significant differences were observed in the basal diameter, total height, and number of leaves of nursery plants across the evaluated localities. Seedlings from The Victoria exhibited the highest total height (38.35 cm) and a relatively large basal diameter (0.91 cm), while The Tundo showed the highest average number of leaves (7). In contrast, seedlings from The Argelia consistently showed the lowest values for all traits. These differences were statistically significant ($p \leq 0.05$), as indicated by the distinct letters above each bar in Figure 12, confirming that locality has a notable effect on early vegetative development.

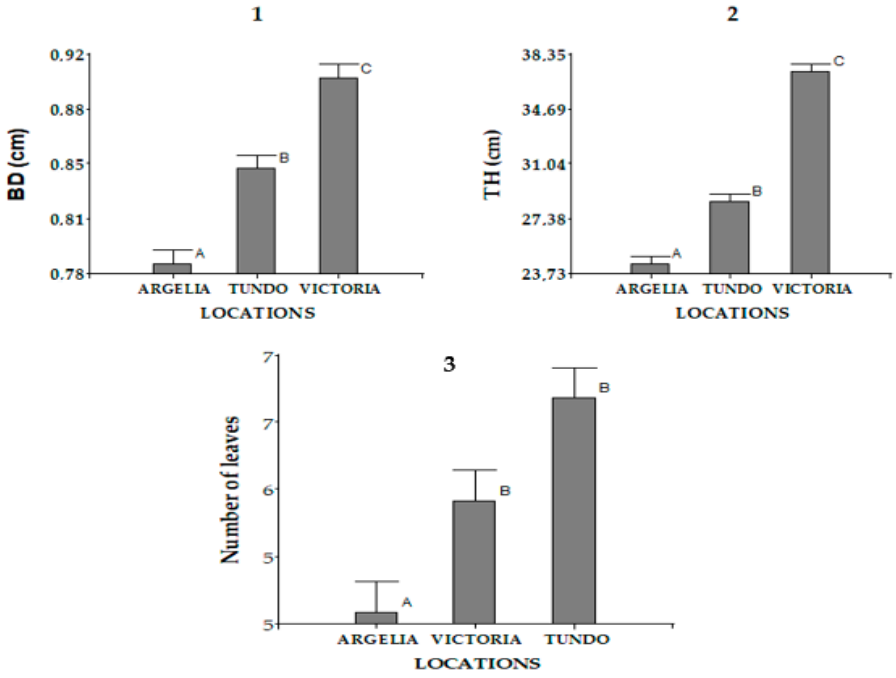


Figure 12. Analysis of variance of basal diameter (**1**), total height (**2**), and number of leaves of the plants by locality (**3**). Different letters indicate significant differences ($p \leq 0.05$).

3.2. Phase II: At the Level of Forest Plantation

3.2.1. Adaptability Parameters

1. Survival %. During the first year of establishment, *J. neotropica* exhibited a high overall survival rate of 92% across the plantation trial. However, stratified and locality-based analyses revealed important differences that highlight environmental influences on early survival.

Among the evaluated strata, the secondary forest showed the highest survival rate (100%), followed by the riparian forest (92%) and pasture (84%). At the locality level, plantations from The Tundo recorded a slightly higher survival rate (94%) than those from The Victoria (90%).

When examining survival by locality within each stratum, more nuanced patterns emerge (Table 7). Both The Tundo and The Victoria achieved 100% survival in the secondary forest. In contrast, the riparian forest showed 96% survival from The Tundo and 88% from The Victoria. The lowest survival percentages occurred in the pasture, with 90% in The Tundo and only 78% from The Victoria.

Table 7. Survival percentage of *J. neotropica* one year after planting by stratum and locality.

Stratum	Locality	Planted	Living	Survival (%)
Secondary Forest	The Tundo	50	50	100
	The Victoria	50	50	100
Subtotal		100	100	100
Riparian Forest	The Tundo	50	48	96
	The Victoria	50	44	88
Subtotal		100	92	92
Pasture	The Tundo	50	45	90
	The Victoria	50	39	78
Subtotal		100	84	84
General Total		300	276	92

Survival percentages recorded during the first year of evaluation for *J. neotropica* plants established in three vegetation types (secondary forest, riparian forest, and pasture) are shown according to fertilization treatment and plant origin (L1: The Tundo; L2: The Victoria).

Plants from L1 (The Tundo) exhibited 100% survival in secondary forest across all treatments, including the unfertilized control (T0), indicating high adaptability to this environment. In contrast, survival rates in riparian forest and pasture under T0 were reduced to 70% and 56%, respectively. The application of NPK significantly improved survival, particularly at 200 and 250 g (T3 and T4), where values exceeded 98% across all cover types. Similarly, urea was highly effective from 150 g onwards (T6–T8), achieving 100% survival in all vegetation types.

For plants from L2 (The Victoria), the response to fertilization was more variable. In the absence of fertilizer (T0), survival dropped notably in pasture (39%) and moderately in riparian forest (67%). The use of NPK led to consistent improvements, especially in secondary and riparian forests, though survival in pasture did not exceed 90%. Urea displayed less stability at intermediate doses, particularly at 150 g (T6), where survival declined in RF (66%) and P (58%). However, higher doses (200 and 250 g; T7 and T8) restored survival to 100% across all cover types (Table 8).

2. Stem straightness at 12 months. It is important to note that the bifurcation caused by the borer in the planted individuals is not considered a genetic phenotypic trait of the species *J. neotropico* (Table 9).

Table 8. Survival percentage of *J. neotropica* plants by ecological stratum and locality.

F1: Localities	F2: Fertilizer	Levels	Treatment	Survival %		
				SF	RF	P
L1: The Tundo	Fert 0: 0	**L1**	T0	100	70	56
	Fert 1: NPK (13-40-13)	**NPK 1:** 100 gr	T1	100	96	95
		NPK 2: 150 gr	T2	100	100	93
		NPK 3: 200 gr	T3	100	100	98
		NPK 4: 250 gr	T4	100	100	99
	Fert 2 = Urea	**Urea:** 100 gr	T5	100	95	65
		Urea: 150 gr	T6	100	100	100
		Urea: 200 gr	T7	100	100	100
		Urea: 250 gr	T8	100	100	100
L2: The Victoria	Fert 0: 0	**L2**	T0	100	67	39
	Fert 1: NPK (13-40-13)	**NPK 1:** 100 gr	T1	100	92	90
		NPK 2: 150 gr	T2	100	80	85
		NPK 3: 200 gr	T3	100	90	90
		NPK 4: 250 gr	T4	100	100	89
	Fert 2 = Urea	**Urea:** 100 gr	T5	100	100	55
		Urea: 150 gr	T6	100	66	58
		Urea: 200 gr	T7	100	100	100
		Urea: 250 gr	T8	100	100	100

Table 9. Percentage of stem shape categories at 12 months for *J. neotropica* by stratum and locality.

Category	No. of Individuals	Sinous (%)	Inclined (%)	Forked (%)	Straight (%)
Strata					
Secondary Forest	100	0.00	0.00	0.00	100.00
Riparian Forest	99	0.00	0.00	0.00	99.00
Pasture	100	0.00	0.00	0.00	100.00
Localities					
The Tundo	150	0.00	0.00	0.00	100.00
The Victoria	149	0.00	0.00	0.67	99.33

Across the three evaluated strata (secondary forest, riparian forest, and pasture), nearly all individuals exhibited straight stems, with 100.00% in both the secondary forest and pasture and 99.00% in the riparian forest.

No individuals with sinuous, inclined, or forked stem shapes were recorded in any of the strata.

Regarding provenance, individuals from The Tundo exhibited a 100% straight stem rate.

In contrast, individuals from The Victoria showed a slightly lower percentage, with 99.33% presenting straight stems and a single individual (0.67%) classified as forked. It is important to note that the bifurcation caused by the borer in the planted individuals is not considered a genetic phenotypic trait of the species *J. neotropica*.

These observations reflect a consistently high occurrence of straight stems across all planting conditions and localities by the 12-month evaluation point.

3.2.2. Phenotypic Characteristics Based on Dasometric Variables

Basal diameter (Bd mm) and total height (Th cm): The average basal diameter and total height are presented for plants from different localities of origin, evaluated across various strata. Among the evaluated localities, The Tundo consistently exhibited the highest mean basal diameters across all three strata, along with the tallest average plant heights (Table 10).

Table 10. Growth of the dasometric variables diameter and height across all strata by locality.

Localities	Strata	Variable	Min	Mean	Max	SD	CV (%)
The Tundo	Secondary Forest	Diameter (mm)	6.39	11.47	20.75	2.87	15.04
		Height (cm)	32.00	54.74	101.00	15.04	27.48
	Riparian Forest	Diameter (mm)	6.83	8.81	11.08	1.12	12.71
		Height (cm)	17.00	49.25	67.00	11.00	22.34
	Pasture	Diameter (mm)	6.24	12.32	20.67	2.68	21.75
		Height (cm)	14.00	41.32	74.00	14.25	34.49
The Victoria	Secondary Forest	Diameter (mm)	6.12	12.08	19.77	3.66	30.30
		Height (cm)	28.00	54.89	108.00	19.97	36.38
	Riparian Forest	Diameter (mm)	5.70	9.57	16.25	2.15	22.47
		Height (cm)	20.00	47.60	87.00	14.80	31.09
	Pasture	Diameter (mm)	5.85	13.86	20.45	3.20	23.09
		Height (cm)	20.00	44.58	76.00	14.29	32.05

Variables represent basal diameter (Diameter) in millimeters and total height (Height) in centimeters, measured 12 months after planting. SD = standard deviation; CV (%) = coefficient of variation.

The average values of the dasometric variables Bd (mm) and Th (cm) are summarized according to strata, localities, and treatments.

Statistically significant differences between strata were found for the mean growth of the dasometric variables, with $p \leq 0.05$ for both diameter and height.

On the other hand, it can be observed that there are no statistically significant differences between the mean growth values of the dasometric variables under study with respect to localities, yielding a p-value of 0.2587 for diameter and a p-value of 0.5681 for height.

Finally, the fertilization treatments applied to the three strata also show no statistically significant differences among them, yielding a p-value of 0.1692 for diameter and 0.7183 for height (Table 11).

Table 12 summarizes the interaction effects between locality, fertilization treatment, and stratum on basal diameter and total height of *J. neotropica* plants.

Table 11. ANOVA of dasometric variables by locality, strata, and treatments.

Category	Factor	Diameter (mm)	SE (mm)	CV Diameter (%)	*p*-Value	Height (cm)	SE (cm)	CV Height (%)	*p*-Value
Locality	The Tundo	11.80	0.27	49.29	0.2587	49.29	1.38	2.8	0.5681
	The Victoria	11.35	0.29	48.14		48.14	1.46	3.03	
Stratum	Secondary Forest	11.78	0.29	54.81	0.0001	54.81	1.55	2.83	0.0001
	Riparian Forest	9.33	0.35	48.13		48.13	1.88	3.91	
	Pasture	13.02	0.30	42.8		42.80	1.59	3.71	
Treatment	T0 (Control)	10.89	0.44	46.32	0.1692	46.32	2.27	4.9	0.7183
	T1 (100 g)	10.91	0.59	49.78		49.78	3.04	6.11	
	T2 (150 g)	11.79	0.61	49.85		49.85	3.15	6.32	
	T3 (200 g)	12.54	0.61	50.15		50.15	3.15	6.28	
	T4 (250 g)	11.68	0.67	50.22		50.22	3.41	6.79	
	T5 (100 g)	10.62	0.63	44.37		44.37	3.21	7.23	
	T6 (150 g)	12.09	0.60	48.61		48.61	3.09	6.36	
	T7 (200 g)	12.19	0.63	52.94		52.94	3.21	6.06	
	T8 (250 g)	12.34	0.64	48.88		48.88	3.27	6.69	

Table 12. Interaction between locality, fertilization treatment, and stratum (CODE) on basal diameter (BD) and total height (TH) of *J. neotropica* plants. Each CODE represents a unique treatment × locality × stratum combination. Tukey groups (α = 0.05) indicate statistically significant differences. For complete statistical data across all 55 combinations, refer to Supplementary Table S1.

Code	Locality	Treatment	Stratum	BD (mm)	SE	Tukey Group (BD)	TH (cm)	SE	Tukey Group (TH)
L2 T3–RF	L2	T3	Riparian forest	7.58	1.56	A	47.00	8.73	A–B
L1 T6–RF	L1	T6	Riparian forest	7.74	2.69	A	51.00	15.10	A–B
L1 T1–RF	L1	T1	Riparian forest	8.11	1.91	A–B	26.00	10.67	A
L2 T5–P	L2	T5	Pasture	16.66	1.21	C	45.80	6.75	A–B
L2 T0–P	L2	T0	Pasture	15.89	1.35	B–C	60.75	7.55	A–B
L1 T0–SF	L1	T0	Secondary forest	13.07	1.21	A–B–C	72.56	6.75	B
L2 T8–SF	L2	T8	Secondary forest	13.14	0.85	A–B–C	52.60	4.77	A–B
L1 T1–SF	L1	T1	Secondary forest	11.07	1.21	A–B–C	66.40	6.75	A–B
L2 T3–SF	L2	T3	Secondary forest	13.31	1.21	A–B–C	69.40	6.75	A–B
L2 T4–SF	L2	T4	Secondary forest	9.23	1.21	A–B–C	42.20	6.75	A–B

Note: Means with the same letter are not significantly different according to Tukey's HSD test (α = 0.05). SE = standard error; BD = basal diameter; TH = total height.

4. Discussion

4.1. Phase I: Influence of Seed Provenance on Early Performance of Juglans neotropica Diels in the Nursery

The results obtained in Phase I reveal a clear effect of seed provenance on various physiological and dasometric traits of *J. neotropica.* In terms of seed quality, high levels of genetic purity were recorded across all provenances: 98.92% for The Tundo, 99.12% for The Victoria, and 98.96% for The Argelia, aligning with values reported by [18] (97.66%). These figures indicate that the seeds were largely free of impurities, supporting effective sexual propagation and the preservation of desirable genetic traits for silvicultural or restoration purposes.

Variations in seed density per unit of mass were also observed: The Tundo presented 30 seeds kg^{-1}, The Victoria 32 seeds kg^{-1}, and The Argelia 35 seeds kg^{-1}. These figures are consistent with reports by [18,19], who documented between 34 and 60 seeds kg^{-1}.

The variability observed among provenances suggests underlying genetic differences with direct and practical implications for forest nursery planning and seed lot selection. From an operational standpoint, the results—particularly those related to growth and survival by locality of origin—allow for the identification of seed sources with superior performance under specific site conditions. This is critical for optimizing production resources, designing tailored fertilization regimes, and adjusting nursery management schedules accordingly. As highlighted by [20], the genetic origin of planting material has a direct influence on its adaptability and field establishment potential, making provenance selection a key step in nursery operations. Complementarily, ref. [21] emphasizes that taking provenance-specific responses into account during nursery phases enhances the compatibility of planting stock with reforestation objectives. The findings of this study reinforce the need to use genetically adapted seed lots for each environment to maximize silvicultural efficiency and ensure the successful production and establishment of *J. neotropica*. These considerations should be integrated into future restoration and forest management programs involving the propagation and deployment of native species such as *J. neotropica*.

During storage, a drop of more than 2% in seed moisture content was recorded after six months at 15–18 °C, compromising seed viability. These findings are supported by [18], who emphasized the critical role of initial seed moisture for germination.

The pre-germination treatments applied—primarily physical and substrate-related—enhanced the uniformity and germination percentage of *J. neotropica* seeds, particularly when compared to the variability observed in the control (T0). Although viability declined after six months of storage, the implemented techniques helped partially mitigate this effect. Previous studies have confirmed that non-chemical pre-germination strategies can improve germination efficiency in native forest species [21,22]. Additionally, ref. [23] emphasized that morphological and biochemical seed evaluation is essential for understanding germination behavior and designing species-specific nursery protocols. In this context, the findings of the present study reinforce the value of simple physical treatments as a practical tool for improving nursery performance when working with limited-quality seed lots.

Germination behavior also varied among provenances and responded differently to pre-sowing treatments. This study observed a germination range consistent with previous reports in the genus *Juglans*, with percentages between 40% and 80% [19,24]. These findings reinforce the importance of tailoring propagation strategies to the genetic origin of the seed material.

In terms of seedling development, The Victoria showed the highest values in both average height (37.20 cm) and basal diameter (9 mm) after six months, followed by The Tundo (28.56 cm, 8 mm) and The Argelia (24.39 cm, 7 mm), which exhibited contrasting growth responses, which align with the provenance-specific patterns previously reported by [25,26]. These studies describe how seed origin influences early vegetative traits such as basal diameter and total height, with certain localities consistently outperforming others under similar nursery conditions. As for foliar development, The Tundo exhibited the highest average number of leaves (7), compared with The Victoria (6) and The Argelia (5), which may indicate initial growth vigor linked to local environmental or genetic factors.

Furthermore, seed size variability—also documented by [27–29]—could be attributed not only to inherent genetic characteristics but also to ecological pressures and dispersal mechanisms, underscoring its importance in shaping early seedling performance. Larger seeds from The Tundo accumulate more reserves, enhancing germination and early seedling

vigor, while smaller seeds from The Argelia may be better adapted for greater dispersal in their environment. This contrast may reflect divergent adaptive strategies shaped by local environmental conditions.

Together, these results highlight that seed provenance is a determining factor for seed quality, germination potential, and early seedling growth of *J. neotropica*, offering essential insights for seed sourcing decisions in forest production and restoration programs.

4.2. Phase II: Influence of Ecosystem and Provenance on the Establishment of J. neotropica in Field Conditions

The establishment success of *J. neotropica* was closely linked to both the type of ecosystem and the provenance of the seedlings. Survival rates were remarkably high across sites, with a total average of 92%, aligning with previous findings such as those reported by [30], who documented a 99.40% survival rate under similar conditions. Notably, survival reached 100% in secondary forests, 92% in riparian forests, and declined to 84% in pastures. This gradient highlights the protective role that forest cover may offer in early plantation stages. These findings reinforce the notion that forested environments, particularly secondary forests, offer more conducive conditions for early species establishment. This suggests greater adaptability of *J. neotropica* under forested conditions, likely due to favorable edaphic and microclimatic factors.

The slightly higher survival rate of plants from The Tundo (94%) compared to The Victoria (90%) may reflect better site adaptation. Ref. [31] found that climatic similarity between seed source and planting site significantly influences performance in tropical pines. Thus, The Tundo's ecological affinity with the local conditions could explain its greater stress tolerance and superior outcome.

Stem form was another key indicator of early vigor. Straightness—a desirable silvicultural trait—was particularly pronounced, with The Tundo reaching 100% of individuals displaying straight, dominant stems, and The Victoria registering 99.33%. It is worth noting that these values may partly reflect a selection effect, as the most vigorous individuals were chosen for outplanting. Nonetheless, the consistently high proportion of straight stems across provenances suggests that this trait may also be influenced by genetic factors and initial morphological quality expressed at the nursery stage.

This pattern highlights the silvicultural relevance of stem architecture and reinforces the importance of parental selection in breeding and domestication programs. While the straight stems observed in The Tundo and The Victoria may reflect both provenance effects and the selection of vigorous individuals at the nursery stage, the results do not allow us to distinguish genetic heritability from environmental or management influences. Nonetheless, these findings are consistent with those of [32], who emphasized the direct relationship between stem form and wood quality.

In terms of growth performance, the basal diameter and total height of plants were influenced by both stratum and provenance. Individuals from The Tundo reached an average diameter of 11.80 mm and 49.29 cm in height, while those from The Victoria showed slightly lower values (11.35 mm and 48.14 cm, respectively).

While these figures are consistent with reports by [30] (12.42 mm Bd; 39.68 cm Th), it was observed that plants grown in open pastures showed reduced growth compared to those in secondary or riparian forests. This is likely due to increased exposure to radiation, limited soil cover, and competition with grasses. Ref. [25] also reported that seedlings surrounded by taller vegetation exhibited better height and diameter growth, likely due to moderated microclimates and reduced competition. These results highlight the importance of considering the interactions between the origin of the plant material and the applied treatments, especially when pre-germination methods are not used, as inherent differences in seed quality or adaptation among localities may become evident.

Collectively, these findings underscore the importance of carefully selecting planting environments and seed sources. Forested sites—particularly secondary and riparian forests—promote better survival and early development, while provenances such as The Tundo may offer advantages in structural form and growth under diverse conditions. Such insights are crucial for designing effective silvicultural strategies and optimizing reforestation outcomes with *J. neotropica* [33].

5. Conclusions

The high physical purity percentage of *Juglans neotropica* Diels seeds (98%) confirms efficient handling and processing, promoting the production of certified seeds and the planning of reforestation programs.

Seed viability is maintained for up to six months but declines with moisture loss exceeding 2%. Implementing optimal storage conditions with controlled temperature and humidity is essential to extending viability beyond this period.

Germination rates vary depending on seed origin and environmental conditions, highlighting the need to select seeds with higher adaptive potential. Additionally, the highest germination percentages for *J. neotropica* were obtained through the control treatment (no pre-germination), immersion in boiling water, mechanical cracking with a hammer, and immersion in water with sunlight exposure. All showed statistically significant differences compared to the other methods evaluated.

However, their effectiveness varied depending on the seed provenance, highlighting an interaction between treatment and locality of origin. This underscores the need to tailor propagation strategies to both the biological characteristics of the seed and the applied treatment. Additionally, nursery conditions—partial shade, controlled irrigation, and well-drained substrate—proved essential for successful seedling establishment. These findings provide practical guidelines for optimizing propagation protocols in restoration and forest production programs.

J. neotropica demonstrated strong establishment capacity during the first year of planting, with an overall survival rate of 92%. The highest survival occurred in secondary forest conditions (100%), suggesting that this environment provides optimal conditions for early establishment. In comparison, riparian forest and grassland sites showed slightly lower survival rates (92% and 84%, respectively), indicating that microenvironmental factors significantly influence planting success. These findings support prioritizing forested sites—particularly secondary forests—for restoration efforts involving this species.

Fertilization played a decisive role in the establishment of *J. neotropica*, particularly under unfavorable conditions such as grasslands. The most effective doses were 200 g and 250 g per plant of both NPK (13-40-13) and urea, which achieved 100% survival across all evaluated sites. These results demonstrate that appropriate fertilization not only enhances early survival but also mitigates site limitations related to soil and microclimate. Therefore, strategic fertilization should be considered an essential practice in ecological restoration and reforestation programs involving this species.

J. neotropica exhibited excellent structural quality at 12 months of establishment, with 99%–100% of individuals displaying straight stems across all evaluated strata and localities. No sinuous or inclined individuals were recorded, and forked stems were virtually absent (0.67% in only one site). These results indicate that, under proper management conditions, this species has strong potential for high-quality timber production, reinforcing its value in both ecological restoration and productive reforestation programs.

Growth in basal diameter and total height was highest in secondary forests and from The Tundo, suggesting better adaptation of the plants from this locality. However, statistical

analysis indicated no significant differences between localities or fertilization treatments. highlighting the importance of site conditions in species development.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f16071141/s1, Table S1: Complete statistical dataset showing the interaction between locality, fertilization treatment, and stratum on basal diameter (BD) and total height (TH) of *J. neotropica* plants across 55 treatment combinations. Includes means, standard errors, and Tukey groupings ($\alpha = 0.05$) for each combination.

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