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Growth of Clones of *Eucalyptus urophylla* in Two Contrasting Soil Conditions in Plantations of Southeastern Mexico

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Abstract: *Eucalyptus urophylla* is important for the establishment of commercial forest plantations in Mexico. Genetic improvement programs are currently being implemented to increase timber productivity. The objectives of this study were to evaluate the adaptability and growth stability of 26 clonal lines of *E. urophylla* in Acrisol and Fluvisol soils and to identify the most suitable genotypes for each soil type. Tree survival, diameter at breast height, and total height were measured annually for six years. These variables were used to estimate individual volume, volume per hectare, and mean annual (MAIv) and current annual (CAIv) volume increment. Survival ranged from 14 to 100% in the Acrisol soil and from 0 to 89% in the Fluvisol soil. Volume per hectare ranged from 65.3 to 488.7 m^3, MAIv from 11.1 to 83.1 $m^3\ ha^{-1}\ year^{-1}$, and CAIv from 2.4 to 134.7 $m^3\ ha^{-1}\ year^{-1}$. Individual heritability (H_i^2) was moderate (0.29–0.49) while the mean heritability of the cloned lines was high (0.73–0.90), indicating that growth is subject to high genetic control. Diameter, height, and volume presented no genotype × environment interaction effects, demonstrating stability in the growth of the clonal lines in both soil types.

Keywords: forest genetic improvement; forest plantations; clonal silviculture; genetic parameters

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1. Introduction

Eucalyptus urophylla S.T. Blake is an important species for the establishment of commercial forest plantations in tropical and subtropical regions worldwide [1]. In Mexico, the species has successfully adapted to regions with warm climates and has been planted for two decades in southern Veracruz, Tabasco, and Oaxaca [2].

Since 2004, genetic improvement programs have focused on improving the productivity of the species in southeastern Mexico [3]. As a result of these programs, most of the current plantations of *E. urophylla* are created with clones of locally selected trees. This process has made it possible to increase the quantity and quality of timber, reaching mean annual volume increment (MAIv) values of 35 $m^3\ ha^{-1}\ year^{-1}$ [4].

In these programs, genetically superior *E. urophylla* trees have been phenotypically selected but not tested in clonal trials to validate their superiority [2]. Previous studies in southeastern Mexico show that several factors, including germplasm origin (seeds, clones) [3], soil texture [5,6], and water and nutrient availability [7], affect the growth in diameter at breast height (DBH) and the total height (H) of the species, such that the performance of clonal lines can vary significantly from one plantation area to another [8].

In addition, climatic factors influence gene expression, altering the phenotypic pattern over time [9]. In this sense, the clonal lines previously adapted to a plantation area might no longer be adapted [10]. Genetic improvement programs should therefore consider the effects of genotype, environment, and the genotype × environment interaction [11]. This latter interaction is important for the definition of improvement zones since it indicates the best genotypes for each environment [12] as well as the most stable genotypes in different environments [13].

It is estimated that the current demand for *E. urophylla* wood to supply the MDF industry in southeastern Mexico can be met by harvesting 3000 hectares annually, with a tendency to increase in the coming years since it is also used to a lesser extent as sawtimber [2]. This will only be achieved if *E. urophylla* plantations are established with the most adaptable and productive genotypes for each plantation area.

The objectives of this study were to evaluate the adaptability and stability of 26 clonal lines of *E. urophylla* in Acrisol and Fluvisol soils in terms of growth and productivity, to select the top five genotypes most suitable for timber production in each soil type, and to determine the magnitude of production by considering the clonal lines with the lowest and highest production among the five clonal lines selected, relative to the current production.

2. Materials and Methods

2.1. Study Area

The study was conducted in a replicated *E. urophylla* trial in two soils in Huimanguillo, Tabasco, in southeastern Mexico (Figure 1). According to Palma-López et al. [14], the first plantation is in an Acrisol soil, the main characteristics of which are that it is weathered, leached, and acidic, with a cation exchange capacity (CEC) of less than 24 cmol (+) kg^{-1}; it is classed as a nutrient-deficient soil. The site is located at 17°42′47.30″ N and 93°36′41.50″ W. The second plantation is in a Fluvisol soil, derived from fluvial sediments, of medium texture, with poor development but good drainage; this soil is rich in nutrients and organic material. The site is located at 17°41′20.50″ N and 93°29′46.80″ W.

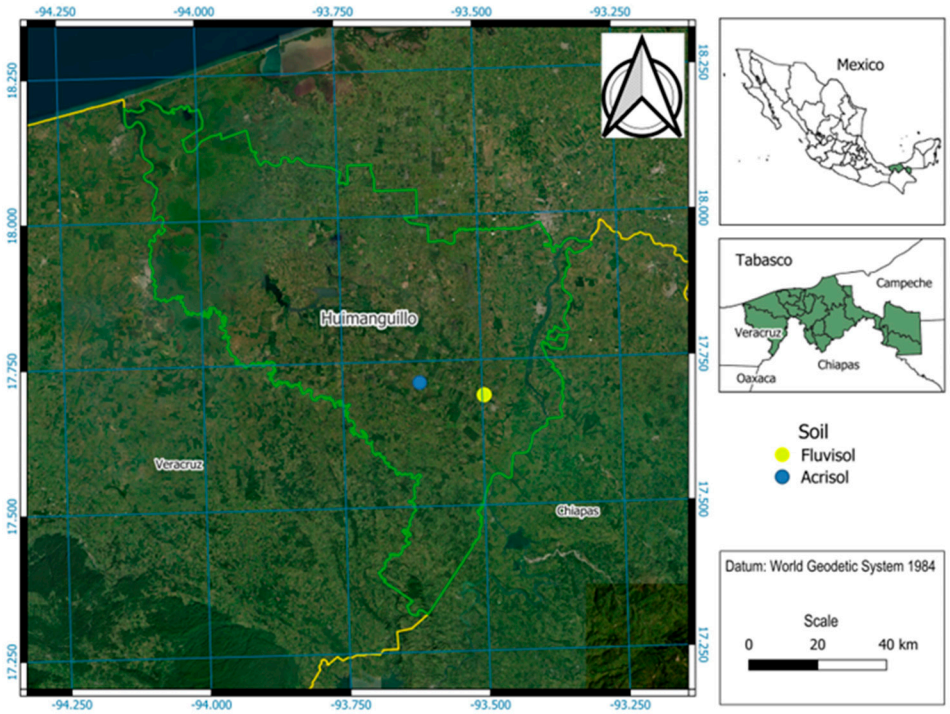

Figure 1. Location of the study area.

The climate is warm and humid with rains throughout the year (Af) [15]. The average annual rainfall is 2200 mm, while the average annual temperature is around 27 °C at both sites.

2.2. Site Preparation

The preparation of the site consisted of scattering leaves and branches of the trees harvested from the previous plantation over the soil. Subsoiling was carried out to a depth of 60 cm to eliminate compaction and to encourage water infiltration and root development. In addition, the plants were placed on a ridge to reduce mortality due to excess water during the rainy season.

2.3. Establishment and Management of the Trial

Twenty-six clonal lines selected from plantations in western Tabasco and southern Veracruz were used for the study. They were selected because they were fast-growing, healthy, and straight-stemmed. In each soil type, a randomized complete block experimental design was used (Figure 2), with six replicates and six plants of each clonal line per plot and a spacing of 3.7 m between rows × 1.9 m between plants, which was equivalent to 1400 trees per hectare.

Column:	1	2	3	4	5	6	7	8	9	10	11	12	13		14	15	16	17	18	19	20	21	22	23	24	25	26
264 border trees																											
Row																											
Rep 1 1	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	Rep 6 1	F9	F8	F3	F13	F22	F24	F19	F21	F18	F6	F12	F17	F2
Rep 1 2	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	Rep 6 2	F9	F8	F3	F13	F22	F24	F19	F21	F18	F6	F12	F17	F2
Rep 1 3	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	Rep 6 3	F9	F8	F3	F13	F22	F24	F19	F21	F18	F6	F12	F17	F2
Rep 1 4	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	Rep 6 4	F9	F8	F3	F13	F22	F24	F19	F21	F18	F6	F12	F17	F2
Rep 1 5	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	Rep 6 5	F9	F8	F3	F13	F22	F24	F19	F21	F18	F6	F12	F17	F2
Rep 1 6	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	Rep 6 6	F9	F8	F3	F13	F22	F24	F19	F21	F18	F6	F12	F17	F2
	1	2	3	4	5	6	7	8	9	10	11	12	13		156	155	154	153	152	151	150	149	148	147	146	145	144
Rep 1 7	F26	F25	F24	F23	F22	F21	F20	F19	F18	F17	F16	F15	F14	Rep 6 1	F16	F26	F10	F14	F1	F25	F11	F4	F20	F5	F23	F15	F7
Rep 1 8	F26	F25	F24	F23	F22	F21	F20	F19	F18	F17	F16	F15	F14	Rep 6 2	F16	F26	F10	F14	F1	F25	F11	F4	F20	F5	F23	F15	F7
Rep 1 9	F26	F25	F24	F23	F22	F21	F20	F19	F18	F17	F16	F15	F14	Rep 6 3	F16	F26	F10	F14	F1	F25	F11	F4	F20	F5	F23	F15	F7
Rep 1 10	F26	F25	F24	F23	F22	F21	F20	F19	F18	F17	F16	F15	F14	Rep 6 4	F16	F26	F10	F14	F1	F25	F11	F4	F20	F5	F23	F15	F7
Rep 1 11	F26	F25	F24	F23	F22	F21	F20	F19	F18	F17	F16	F15	F14	Rep 6 5	F16	F26	F10	F14	F1	F25	F11	F4	F20	F5	F23	F15	F7
Rep 1 12	F26	F25	F24	F23	F22	F21	F20	F19	F18	F17	F16	F15	F14	Rep 6 6	F16	F26	F10	F14	F1	F25	F11	F4	F20	F5	F23	F15	F7
	26	25	24	23	22	21	20	19	18	17	16	15	14		131	132	133	134	135	136	137	138	139	140	141	142	143
Rep 2 13	F3	F10	F12	F4	F1	F24	F19	F15	F18	F26	F5	F16	F8	Rep 5 1	F21	F16	F8	F24	F10	F26	F3	F14	F11	F2	F17	F15	F1
Rep 2 14	F3	F10	F12	F4	F1	F24	F19	F15	F18	F26	F5	F16	F8	Rep 5 2	F21	F16	F8	F24	F10	F26	F3	F14	F11	F2	F17	F15	F1
Rep 2 15	F3	F10	F12	F4	F1	F24	F19	F15	F18	F26	F5	F16	F8	Rep 5 3	F21	F16	F8	F24	F10	F26	F3	F14	F11	F2	F17	F15	F1
Rep 2 16	F3	F10	F12	F4	F1	F24	F19	F15	F18	F26	F5	F16	F8	Rep 5 4	F21	F16	F8	F24	F10	F26	F3	F14	F11	F2	F17	F15	F1
Rep 2 17	F3	F10	F12	F4	F1	F24	F19	F15	F18	F26	F5	F16	F8	Rep 5 5	F21	F16	F8	F24	F10	F26	F3	F14	F11	F2	F17	F15	F1
Rep 2 18	F3	F10	F12	F4	F1	F24	F19	F15	F18	F26	F5	F16	F8	Rep 5 6	F21	F16	F8	F24	F10	F26	F3	F14	F11	F2	F17	F15	F1
	27	28	29	30	31	32	33	34	35	36	37	38	39		130	129	128	127	126	125	124	123	122	121	120	119	118
Rep 2 19	F17	F2	F6	F20	F9	F13	F21	F14	F25	F11	F22	F23	F7	Rep 5 1	F13	F7	F18	F20	F4	F25	F6	F12	F9	F23	F19	F22	F5
Rep 2 20	F17	F2	F6	F20	F9	F13	F21	F14	F25	F11	F22	F23	F7	Rep 5 2	F13	F7	F18	F20	F4	F25	F6	F12	F9	F23	F19	F22	F5
Rep 2 21	F17	F2	F6	F20	F9	F13	F21	F14	F25	F11	F22	F23	F7	Rep 5 3	F13	F7	F18	F20	F4	F25	F6	F12	F9	F23	F19	F22	F5
Rep 2 22	F17	F2	F6	F20	F9	F13	F21	F14	F25	F11	F22	F23	F7	Rep 5 4	F13	F7	F18	F20	F4	F25	F6	F12	F9	F23	F19	F22	F5
Rep 2 23	F17	F2	F6	F20	F9	F13	F21	F14	F25	F11	F22	F23	F7	Rep 5 5	F13	F7	F18	F20	F4	F25	F6	F12	F9	F23	F19	F22	F5
Rep 2 24	F17	F2	F6	F20	F9	F13	F21	F14	F25	F11	F22	F23	F7	Rep 5 6	F13	F7	F18	F20	F4	F25	F6	F12	F9	F23	F19	F22	F5
	52	51	50	49	48	47	46	45	44	43	42	41	40		105	106	107	108	109	110	111	112	113	114	115	116	117
Rep 3 25	F15	F4	F10	F7	F11	F20	F19	F18	F5	F26	F23	F25	F8	Rep 4 1	F1	F15	F7	F14	F13	F5	F19	F6	F16	F24	F4	F8	F21
Rep 3 26	F15	F4	F10	F7	F11	F20	F19	F18	F5	F26	F23	F25	F8	Rep 4 2	F1	F15	F7	F14	F13	F5	F19	F6	F16	F24	F4	F8	F21
Rep 3 27	F15	F4	F10	F7	F11	F20	F19	F18	F5	F26	F23	F25	F8	Rep 4 3	F1	F15	F7	F14	F13	F5	F19	F6	F16	F24	F4	F8	F21
Rep 3 28	F15	F4	F10	F7	F11	F20	F19	F18	F5	F26	F23	F25	F8	Rep 4 4	F1	F15	F7	F14	F13	F5	F19	F6	F16	F24	F4	F8	F21
Rep 3 29	F15	F4	F10	F7	F11	F20	F19	F18	F5	F26	F23	F25	F8	Rep 4 5	F1	F15	F7	F14	F13	F5	F19	F6	F16	F24	F4	F8	F21
Rep 3 30	F15	F4	F10	F7	F11	F20	F19	F18	F5	F26	F23	F25	F8	Rep 4 6	F1	F15	F7	F14	F13	F5	F19	F6	F16	F24	F4	F8	F21
	53	54	55	56	57	58	59	60	61	62	63	64	65		104	103	102	101	100	99	98	97	96	95	94	93	92
Rep 3 31	F2	F9	F12	F16	F21	F24	F22	F6	F17	F14	F3	F13	F1	Rep 4 1	F18	F22	F17	F12	F25	F26	F2	F9	F10	F11	F3	F23	F20
Rep 3 32	F2	F9	F12	F16	F21	F24	F22	F6	F17	F14	F3	F13	F1	Rep 4 2	F18	F22	F17	F12	F25	F26	F2	F9	F10	F11	F3	F23	F20
Rep 3 33	F2	F9	F12	F16	F21	F24	F22	F6	F17	F14	F3	F13	F1	Rep 4 3	F18	F22	F17	F12	F25	F26	F2	F9	F10	F11	F3	F23	F20
Rep 3 34	F2	F9	F12	F16	F21	F24	F22	F6	F17	F14	F3	F13	F1	Rep 4 4	F18	F22	F17	F12	F25	F26	F2	F9	F10	F11	F3	F23	F20
Rep 3 35	F2	F9	F12	F16	F21	F24	F22	F6	F17	F14	F3	F13	F1	Rep 4 5	F18	F22	F17	F12	F25	F26	F2	F9	F10	F11	F3	F23	F20
Rep 3 36	F2	F9	F12	F16	F21	F24	F22	F6	F17	F14	F3	F13	F1	Rep 4 6	F18	F22	F17	F12	F25	F26	F2	F9	F10	F11	F3	F23	F20
	78	77	76	75	74	73	72	71	70	69	68	67	66		79	80	81	82	83	84	85	86	87	88	89	90	91

Figure 2. Field assay design. Replicates (Rep); clonal lines (F1–F26); experimental plots (1–156).

The plants were established in the field in November 2015 and evaluated for six years, from 2016 to 2021. During this period, the soil was fertilized twice: first at thirty days after establishment and again when the trial was one year old. In both instances, 250 g of formula 18-15-15 (N-P-K) fertilizer was applied per plant. Weed control was conducted during the first two years of growth. Along the planting line, weeds were cut manually with

a machete, while a tractor fitted with a brush cutter was used between these lines. No pruning or thinning was carried out.

2.4. Evaluation of Growth

At the ages of 1, 2, 3, 4, 5, and 6 years, tree diameter at breast height (DBH) was measured at 1.30 m above the ground with a diametric measuring tape, and total height was measured with an ECII D electronic clinometer (Haglöf, Sweden). Survival of the clonal lines was determined by quantifying the living trees.

With the DBH and total height, the volume per tree was estimated in cubic meters (m^3) using the equation of Hernández-Ramos et al. [16] for *E. urophylla* in Huimanguillo (Equation (1)).

$$V_t = 0.32204 \times (DBH/100)^2 \times H \quad (1)$$

where V_t = volume per tree in cubic meters (m^3); DBH = diameter at breast height in centimeters (cm); and H = total height of the tree in meters (m).

With the average volume per clonal line, the volume per hectare in m^3 was estimated using the equation of Silva et al. [17] (Equation (2)).

$$VOL = (vol)(sup)(trees\ per\ hectare) \quad (2)$$

where VOL = volume per hectare in m^3; vol = mean volume per clonal line in m^3 per tree; sup = decimal equivalent of the percentage survival; and $trees\ per\ hectare$ = the plantation density of the trials.

The mean annual volume increment (*MAIv*) in timber of each clonal line was estimated in m^3 per hectare per year ($m^3\ ha^{-1}\ year^{-1}$) using the equation of Murillo-Brito et al. [18] (Equation (3)).

$$MAIv = \frac{\sum_1^n v}{S \times E} \quad (3)$$

where v = the sum of the volume of n living trees per clonal line in the trial; S = area in hectares occupied by all the trees of each clonal line in the trial; and E = age of the trial in years.

The current annual volume increment (*CAIv*) ($m^3\ ha^{-1}\ year^{-1}$) in timber was calculated as the difference in volume per hectare between the beginning and end of a year of growth.

2.5. Data Analysis

2.5.1. Analysis of Survival

To determine differences in survival between clonal lines, the log-rank test with the Kaplan–Meier method was used (Equation (4)).

$$P_{(t)} = P_r\ (\mathrm{T} > \mathrm{t}) \quad (4)$$

where $P_{(t)}$ = the function of survival estimated in a specific time t; P_r = probability of survival of an individual from the beginning of the study (t) until a given time (T) [19]. The analysis was conducted with the function "survfit" of the "survival" package of R (Rstudio version 2023.09.1+494) [20].

2.5.2. Analysis of Growth

A Shapiro–Wilk test showed that the variables studied over time did not follow a normal distribution ($p < 0.05$). Therefore, to determine statistical differences among clonal lines, age, and the clonal line × age interaction in terms of diameter, height, individual volume, volume per hectare, MAIv, and CAIv, a repeated measures analysis was performed with the transformation of ranks aligned with the "ARTool" package of R [21].

To estimate significant annual differences in the growth variables among the clonal lines, a one-way ANOVA with blocks was conducted, followed by a Tukey test for mean

comparisons when the data were normally distributed and a Friedman test for a complete block design when the distribution of the data was not normal [22].

2.5.3. Estimation of Genetic Parameters

The values of variance for the diameter, height, and volume per hectare were estimated for each soil type separately (Equation (5)), and for both together (Equation (6)), using the restricted maximum likelihood (REML) procedure [23], for which a linear mixed-effects model was fitted using the package "lme4" in R [20]. Clonal line and the interaction genotype × environment were considered as random effects [24], while soil type and replicate were considered as fixed effects [25,26].

$$Y_{ijk} = \mu + \gamma_i + \tau_j + (\gamma\tau_{ij}) + \varepsilon_{ijk} \quad (5)$$

where Y_{ijk} = response variable of the k^{th} tree of the i^{th} replicate and j^{th} clonal line; μ = general mean; γ_i = fixed effect of the i^{th} replicate; τ_j = random effect of the j^{th} clonal line; $(\gamma\tau_{ij})$ = random interaction effect of the i^{th} replicate and the j^{th} clonal line; ε_{ij} = random error corresponding to the observation Y_{ijk}.

$$Y_{ijkl} = \mu + E_i + \gamma_{j(i)} + \tau_k + E\tau_{ik} + \varepsilon_{ijkl} \quad (6)$$

where Y_{ijk} = response variable of the k^{th} clonal line of the j^{th} replicate of the i^{th} environment (soil type); E_i = fixed effect of the i^{th} environment; $\gamma_{j(i)}$ = fixed effect of the j^{th} replicate in the i^{th} environment; τ_k = random effect of the k^{th} clonal line; $E\tau_{ik}$ = random interaction effect of the k^{th} clonal line in the i^{th} environment; ε_{ijkl} = random error corresponding to the observation Y_{ijkl}.

The significance of the random effects was verified with the likelihood ratio test (LRT) using a chi-square test. For the significance of the fixed effects, the F-test was used [27].

With the variances of the clonal line (σ_g^2), the interaction genotype × environment (σ_{ga}^2), and the residual variance (σ_e^2), the following genetic parameters were obtained:

Heritability, in a broad individual sense (H_i^2) (Equation (7)) and of the mean of the clonal lines (H_c^2) (Equation (8)), per soil type [28]:

$$H_i^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2) \quad (7)$$

$$H_c^2 = \sigma_g^2/[\sigma_g^2 + (\sigma_e^2/r)] \quad (8)$$

where r = number of replicates.

Heritability, in a broad individual sense (H_i^2) (Equation (9)) and of the mean of the clonal lines (H_c^2) (Equation (10)), for both soils together [23]:

$$H_i^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ga}^2 + \sigma_e^2) \quad (9)$$

$$H_c^2 = \sigma_g^2/[\sigma_g^2 + (\sigma_{ga}^2/s) + (\sigma_e^2/sb)] \quad (10)$$

where s = number of soil types (2) and b = number of replicates (6).

The standard error (s.e.) of the heritability was estimated using the equation of Becker [29] (Equation (11)):

$$s.e. = \sqrt{\frac{2(n-1)(1-H_c^2)[1+(k-1)H_c^2]^2}{b^2(n-N)(N-1)}} \quad (11)$$

where b = number of replicates; N = number of clonal lines evaluated; and n = total number of individuals evaluated.

The genotypic (CV_g) (Equation (12)), residual (CV_e) (Equation (13)), and relative coefficients of variation (CV_r) (Equation (14)) of the growth traits were estimated using the equations of Makouanzi et al. [30]:

$$CV_g = \frac{\sqrt{\sigma_g^2}}{\mu} \quad (12)$$

$$CV_e = \frac{\sqrt{\sigma_e^2}}{\mu} \quad (13)$$

$$CV_r = CV_g / CV_e \quad (14)$$

where μ is the mean value of the evaluated trait.

The genetic ($r_{g(x,y)}$) (Equation (15)), environmental ($r_{e(x,y)}$) (Equation (16)), and phenotypic ($r_{P(x,y)}$) (Equation (17)) correlations were estimated following the approach of Falconer and Mackay [31]:

$$r_{g(x,y)} = Cov_{g(x,y)} / \sqrt{\sigma_{gx}^2 \times \sigma_{gy}^2} \quad (15)$$

$$r_{e(x,y)} = Cov_{e(x,y)} / \sqrt{\sigma_{ex}^2 \times \sigma_{ey}^2} \quad (16)$$

where $Cov_{g(x,y)}$ and $Cov_{e(x,y)}$ = the genetic covariance and environmental covariance between the correlated traits; σ_{gx}^2 and σ_{ex}^2 = the genetic variance and environmental variance of trait x; and σ_{gy}^2 and σ_{ey}^2 = the genetic variance and environmental variance of trait y.

$$r_{P(x,y)} = H_x H_y r_{g(x,y)} + (1 - H_x)(1 - H_y) r_{e(x,y)} \quad (17)$$

where H_x and H_y = the square root of the mean heritability of the clonal lines of the correlated traits.

Precision of selection (Equation (18)) of Braga et al. [23]:

$$r_{gg} = \sqrt{H_c^2} \quad (18)$$

Mean volume per hectare was used to represent the adaptability, stability, and productivity of the clonal lines using biplot graphs [32] with the "metan" package of Rstudio Version 09.1. [20].

3. Results

3.1. Survival

Since the survival of clonal lines F15 and F17 was 0% in the Fluvisol soil and 14% in the Acrisol soil, these lines were not considered in the growth analysis. Only the growth of 24 clonal lines is reported.

The log-rank test showed significant differences between the survival of the clonal lines in the Acrisol ($\chi^2 = 255$, D.F. = 25, $p < 0.001$) and Fluvisol ($\chi^2 = 316$, D.F. = 25, $p < 0.001$) soils. At the end of the evaluation, survival ranged from 14 to 100% in the Acrisol and from 0 to 89% in the Fluvisol.

3.2. Growth of the E. urophylla Clonal Lines

The differences in growth among the clonal lines were independently generated by genetic constitution and by the particular characteristics of the soils in which the trees were grown, and there was no interaction effect between the clonal lines and soil types. The greater average height of the clonal lines in the Acrisol soil indicates the presence of more suitable conditions for the growth of *E. urophylla* than in the Fluvisol soil.

The repeated measures analysis showed significant differences ($p < 0.001$) among the clonal lines and tree ages in both soil types for all the growth variables measured (total height, diameter, volume, volume per hectare, MAIv, and CAIv), except volume per hectare between the ages of five and six years in the Fluvisol ($p = 0.993$). At the end of the

evaluation, the trees had, on average, greater dimensions in the Acrisol than in the Fluvisol (Table 1).

Notable variations were observed in the average diameter recorded at the end of the study among the different clonal lines, with measurements of 13.9 (F6) and 21.4 (F18) cm in the Acrisol and 12.4 (F11) and 22.9 (F18) cm in the Fluvisol (Table 1). The growth in height of the clonal lines ranged from 17.7 (F3) to 26.9 (F13) m in the sixth year in the Acrisol and from 15.8 (F3) to 21.2 (F13) m in the Fluvisol.

Table 1. Survival, growth in height, diameter, and volume per hectare at six years of age in a clonal trial of *E. urophylla* in two soils in Huimanguillo in Tabasco, Mexico.

Clonal Line	Acrisol Soil				Fluvisol Soil			
	S (%)	H (m)	DBH (cm)	VOL (m^3 ha^{-1})	S (%)	H (m)	DBH (cm)	VOL (m^3 ha^{-1})
F1	47	22.9 abc	18.4 abc	228.4 abcde	50	20.3 abcd	17.6 abcd	150.0 bc
F2	86	21.9 abc	16.0 abc	238.3 abcde	83	20.0 abcd	16.9 abcd	235.5 ab
F3	94	17.7 c	13.7 bc	153.6 bcde	64	15.7 d	13.5 cd	93.8 bc
F4	58	20.5 bc	14.3 abc	122.6 cde	58	18.0 abcd	14.3 abcd	113.1 bc
F5	97	24.0 ab	16.8 abc	315.6 abcd	56	18.6 abcd	16.2 abcd	140.9 bc
F6	53	20.5 bc	13.9 bc	103.8 cde	56	19.1 abcd	13.8 bcd	94.9 bc
F7	83	22.9 abc	18.0 abc	332.3 abcd	44	20.1 abcd	18.9 abcd	165.9 bc
F8	86	21.5 abc	14.6 abc	191.7 abcde	86	16.9 cd	13.7 bcd	135.9 bc
F9	81	21.3 abc	17.7 abc	279.1 abcde	72	18.0 abcd	14.7 abcd	143.2 bc
F10	89	21.4 abc	16.8 abc	274.8 abcde	72	18.9 abcd	16.8 abcd	197.3 abc
F11	78	19.1 bc	14.3 abc	164.1abcde	86	17.2 cd	12.4 d	110.1 bc
F12	89	18.5 bc	15.2 abc	240.3 abcde	56	18.5 abcd	19.7 ab	201.3 abc
F13	64	24.9 ab	19.5 ab	295.7 abcd	58	21.6 a	19.6 abc	211.7 abc
F14	86	21.2 abc	13.5 c	165.0 abcde	86	18.9 abcd	14.4 abcd	150.8 bc
F16	86	21.8 abc	13.7 bc	166.5 abcde	75	18.6 abcd	13.9 bcd	124.5 bc
F18	83	23.7 abc	21.4 a	489.9 a	64	21.6 a	22.9 a	336.6 a
F19	92	22.4 abc	15.0 abc	223.7 abcde	81	19.4 abcd	15.5 abcd	170.7 bc
F20	89	21.4 abc	15.1 abc	199.6 abcde	89	19.6 abcd	15.6 abcd	194.8 abc
F21	100	23.5 abc	18.8 abc	408.0 ab	86	19.4 abcd	17.3 abcd	233.9 ab
F22	50	22.6 abc	16.2 abc	145.3 cde	56	17.6 bcd	14.3 abcd	106.9 bc
F23	83	26.8 a	18.5 abc	346.8 abc	67	20.1 abcd	18.2 abcd	209.7 abc
F24	61	21.6 abc	14.4 abc	142.7 cde	39	18.0 abcd	13.8 bcd	65.3 c
F25	86	22.1 abc	14.9 abc	209.0 abcde	72	18.0 abcd	15.6 abcd	157.7 bc
F26	78	21.3 abc	15.4 abc	235.5 abcde	69	18.3 abcd	16.2 abcd	177.9 bc
Average		21.9	16.0	223.3		18.9	16.1	163.4
SD		2.0	2.1	101.2		1.4	2.5	58.9

S = survival, DBH = diameter at breast height (cm), H = total height (m), VOL = volume per hectare (m^3), SD = standard deviation. Different lowercase letters in a column indicate a significant difference ($p < 0.05$) among means according to the Tukey test.

Growth in diameter was similar in both soil types ($p > 0.05$); only the factor clonal line had a significant effect ($p < 0.001$) (Table 2). Growth in height was influenced by both clonal line ($p < 0.001$) and soil type ($p < 0.01$), and there was no genotype × environment interaction effect on height.

Individual volume (m^3) per clonal line increased during all ages; however, volume per hectare (m^3) decreased with age in some clonal lines when mortality was included. Soil type and clonal line had a significant effect ($p < 0.001$) on growth in volume per hectare, but no genotype × environment interaction effects were observed for this variable (Table 2). Clonal line F18 had the highest timber volume per hectare at the end of the study in both soil types; the difference was 30% higher in the Acrisol than in the Fluvisol (Table 1).

Table 2. Significance of the mean squares associated with fixed effects (environment and genotype × environment) and significance of chi-squared test associated with the random effects (genotype and genotype × environment) at six years of age, in a clonal trial of *E. urophylla* in Huimanguillo in Tabasco, Mexico.

Effect	Test	DBH	H	VOL
Replicate × Environment	F-test	1.609 ^{ns}	3.313 ***	4.67 ***
Environment (soil type)	F-test	0.520 ^{ns}	63.124 **	22.72 ***
Genotype (clonal line)	LRT	24.978 ***	13.605 ***	19.03 ***
Genotype x Environment	LRT	0.020 ^{ns}	0.823 ^{ns}	1.53 ^{ns}

LRT: likelihood ratio test; DBH: diameter at breast height; H: total height; VOL: volume per hectare; *** $p < 0.001$; ** $p < 0.01$; ^{ns} = not significant.

The highest MAIv of the clonal lines in the Acrisol soil occurred between the first and second year of growth ($p < 0.001$) and then tended to remain constant until the end of the evaluation ($p > 0.05$) (Figure 3a). In the Fluvisol soil, the MAIv varied over the six years of study ($p < 0.001$). In this soil, the highest MAIv also occurred between the first and second year of growth; however, it reached its maximum value in the third year for most of the clonal lines, varying from 18.0 (F24) to 53.3 (F13) $m^3\ ha^{-1}\ yr^{-1}$. Subsequently, the MAIv gradually decreased until the sixth year of evaluation (Figure 3b). The growth of clonal line F18 presented a different behavior from that of the others: in the Acrisol, the MAIv kept increasing until the sixth year (Figure 3a), while in the Fluvisol, it reached its maximum value at four years, with 69.1 $m^3\ ha^{-1}\ yr^{-1}$ (Figure 3b).

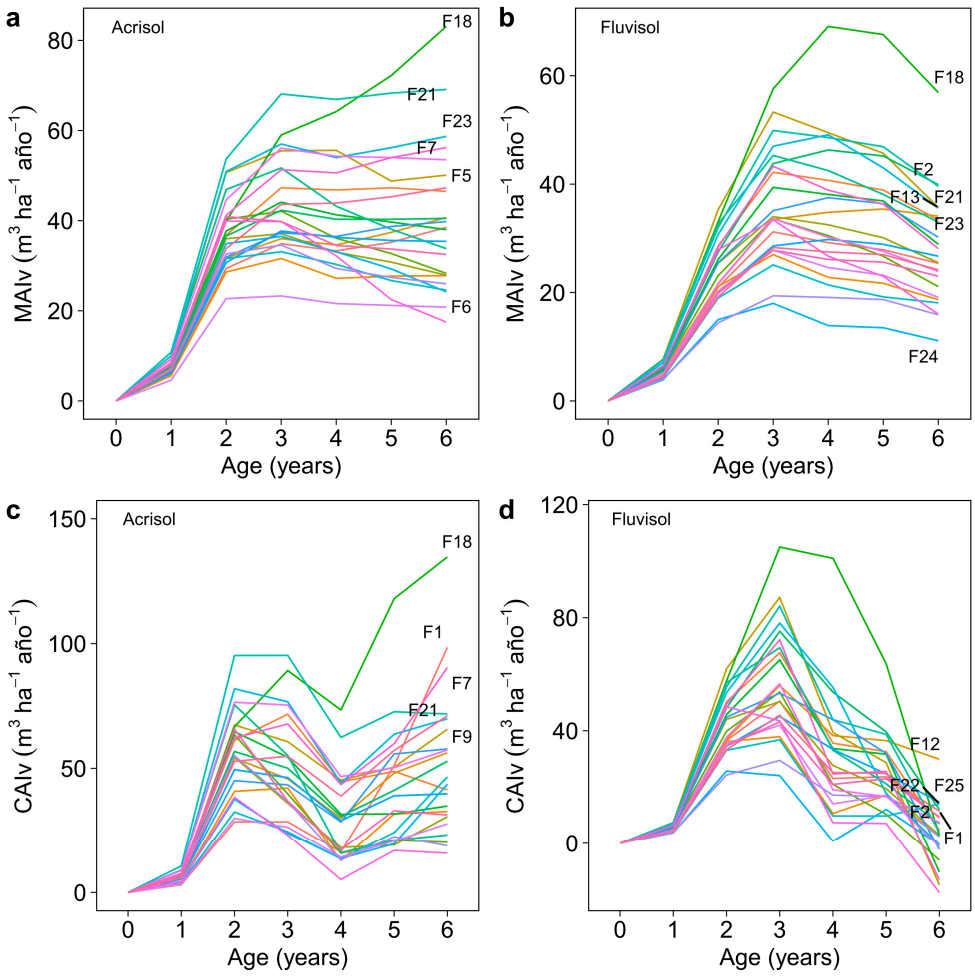

Figure 3. Mean annual increase in volume (MAIv) in the Acrisol (**a**) and Fluvisol (**b**) soils; current annual increase in volume (CAIv) in the Acrisol (**c**) and Fluvisol (**d**) soils.

In the Acrisol, the highest CAIv was presented in the second year in most of the clonal lines, varying from 40.3 (F4) to 95.2 (F21) m^3 ha^{-1} $year^{-1}$. Between the fifth and sixth years of age, some lines underwent an increase in CAIv (Figure 3c). In the Fluvisol, the highest CAIv was recorded in the third year. At this age, it varied from 24.0 (F24) to 105.0 (F18) m^3 ha^{-1} $year^{-1}$. From the third year onwards, the CAIv decreased across all the clonal lines (Figure 3d).

3.3. Genetic Parameters

Residual variance (σ_e^2) tended to be greater than genotypic variance (σ_g^2) in both soil types separately and when considered together (Table 3). The H_i^2 was slightly higher in the Fluvisol than in the Acrisol. On the other hand, the H_c^2 was higher when both soils were considered together. The CV_g and CV_e were consistently higher in volume per hectare, followed by height and diameter in both soil types, when considered separately and together. The estimated values for the precision of selection (r_{gg}) were greater than 0.89, except for height in the Acrisol.

Table 3. Variances and genetic parameters of diameter, height, and volume per hectare in a trial of *E. urophylla* in Huimanguillo in Tabasco, Mexico.

Parameter	Acrisol Soil			Fluvisol Soil			Both Soils Together		
	DBH	***H***	***VOL***	***DBH***	***H***	***VOL***	***DBH***	***H***	***VOL***
σ_g^2	3.86 ***	2.94 ***	7482.94 ***	5.23 ***	1.57 ***	2692.85 ***	4.51 ***	1.99 ***	4600.08 ***
σ_e^2	6.25	6.38	7868.14	5.76	2.38	4657.03	5.93	4.67	6383.33
σ_{ga}^2							0.04 ns	0.21 ns	467.51 ns
H_i^2	0.38 ± 0.10	0.32 ± 0.02	0.49 ± 0.09	0.48 ± 0.10	0.40 ± 0.10	0.37 ± 0.04	0.43 ± 0.08	0.29 ± 0.01	0.40 ± 0.01
H_c^2	0.79 ± 0.06	0.73 ± 0.01	0.85 ± 0.04	0.84 ± 0.05	0.80 ± 0.05	0.78 ± 0.02	0.90 ± 0.03	0.80 ± 0.01	0.86 ± 0.05
CV_g (%)	12.24	7.83	36.60	14.22	6.65	31.75	13.23	6.94	33.93
CV_e (%)	15.57	11.55	37.53	14.94	8.19	41.76	15.17	10.62	39.97
CV_r	0.79	0.86	0.98	0.95	0.81	0.76	0.87	0.65	0.85
r_{gg}	0.89	0.68	0.92	0.92	0.89	0.88	0.95	0.89	0.93

DBH: diameter at breast height; *H*: total height; *VOL*: volume per hectare; σ_g^2: genetic variance; σ_e^2: variance of the error; σ_{ga}^2: variance of the interaction genotype × environment; H_i^2: heritability in a broad sense for clones; H_c^2: heritability in a broad sense for the mean of the clonal lines; CV_g: coefficient of genetic variation; CV_e: coefficient of residual variation; CV_r: coefficient of relative variation; r_{gg}: precision of selection; *** $p < 0.001$; ns: not significant for the chi-squared test.

The genetic and phenotypic correlations among the growth variables were moderate to high. The lowest correlations were between tree height and volume per hectare (Table 4).

Table 4. Genetic (above the diagonal) and phenotypic (below the diagonal) correlations of growth variables in a clonal trial of *E. urophylla* of six years of age.

Trait	Acrisol Soil			Fluvisol Soil			Both Soils Together		
	DBH	***H***	***VOL***	***DBH***	***H***	***VOL***	***DBH***	***H***	***VOL***
DBH		0.69 *	0.90 *		0.80 *	0.91 *		0.80 *	0.99 *
H	0.54 *		0.58 *	0.66 *		0.80 *	0.69 *		0.72 *
VOL	0.74 *	0.46 *		0.74 *	0.64 *		0.87 *	0.60 *	

DBH: diameter at breast height; *H*: total height; *VOL*: volume per hectare. The significance of the correlation is * $p < 0.05$.

The GGE biplot analysis explained 100% of the phenotypic variation (Figure 4). The distance between the environment vectors (blue lines) shows differences between the two test environments (soil types) (Figure 4a). However, the acute angle formed between the vectors indicates a positive correlation between them [11].

The green abscissa (Figure 4b) represents the average environment, and the direction of the arrow indicates the performance of the clonal lines [33]. In this case, clonal line F18 presented the highest growth in volume per hectare, and F24 presented the lowest growth. The dotted line perpendicular to the average environment represents the stability of the clonal lines: the greater the distance from the average environment, the more unstable that the clonal line is in the environment [33]. Lines F13, F10, and F23, which are close to the average environment, are the most stable genotypes in both soil types.

The genotypes that form the polygon (Figure 4c) are those that are the most different from the population average, both positively and negatively, which represents sensitivity to these soil conditions [34,35]. Clonal line F21 grew best in Acrisol soil, F18 was best in both soil types, and F2 grew best in Fluvisol soil. The dotted black lines (Figure 4c) starting from the origin represent the hypothetical number of environments generated by the performance of the clonal lines [35]. Growth in volume per hectare grouped the two soil types in the same sector.

The circle indicated by the black arrow (Figure 4d) represents the ideal genotype for the soil conditions studied [36], and clonal line F18 was the closest to this condition. The increasing distance of the genotypes from each other (larger concentric circles) indicates that their suitability is decreasing. Clonal lines F24 and F6 were the least desirable for both soil types.

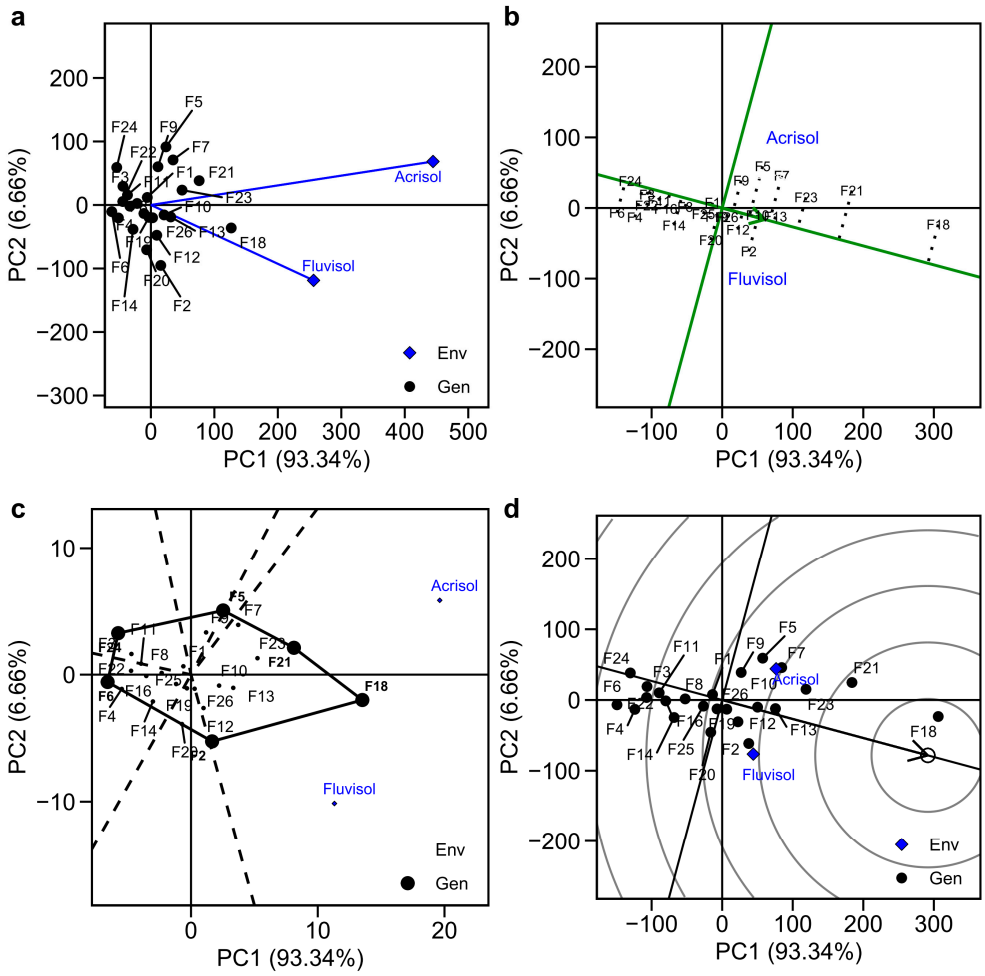

Figure 4. GGE biplot analysis for (**a**) the relationship between soil types; (**b**) the average growth and stability of the clonal lines ("Mean performance vs. Stability"); (**c**) the performance of each clonal line per soil type ("Which-won-where"); and (**d**) the comparison between clonal lines and the "ideal" genotype ("Ranking genotypes").

4. Discussion

The results of this study support the need to evaluate genotypes in clonal trials to determine and obtain those that are genetically superior, rather than selecting trees for their phenotypic characteristics [37], and to test the performance of clonal lines in auto- (clones of the same clonal line) or allo-competition (clones of different clonal line), since the arrangement also affects tree performance [38,39].

The growth observed in our study is comparable with data reported for *E. urophylla* in different parts of the world; the diameters and heights were similar to those reported by Oliveira et al. [40] in clonal lines of *E. urophylla* in Brazil. These authors reported diameters from 9.5 cm to 20.8 cm and heights from 16.9 m to 29.4 m at 6 years of age. Pereira et al. [41] reported diameters of 14.8 to 15.5 cm in clonal lines of *E. urophylla* at 7.5 years of age in Brazil.

The timber volume per hectare (489.9 m^3 ha^{-1}) obtained in clonal line F18 in our study at six years of age is comparable to those of productive plantations worldwide. The result was 63% higher than that reported by Sadono et al. [42] for *E. urophylla* (181.1 m^3 ha^{-1}) in Indonesia at 20 years of age and 15% higher than that reported by Resquin et al. [43] (416.4 m^3 ha^{-1}) for three fast-growing species (*Eucalyptus benthamii* Maiden & Cambage, *Eucalyptus dunnii* Maiden, and *Eucalyptus grandis* Hill ex Maiden) at 4.7 years of age in Uruguay.

Low tree survival caused a decrease in timber volume per hectare, indicating that it is necessary to evaluate clonal lines at different spacings to determine the optimum planting density to maintain a balance between timber production and survival. Several studies report findings related to planting density. Zhao et al. [44] found that, in 12-year-old *Pinus taeda* L. plots in the southern United States, those with 741 trees ha^{-1} had a 93% survival rate and an average diameter of 25 cm, while those with 4448 trees ha^{-1} had a 73% survival rate and an average diameter of 13 cm. Bahru et al. [45] evaluated four planting densities in 6.6-year-old *E. grandis* in Ethiopia, finding no significant differences in survival but showing differences in growth. These results indicate that, in addition to genetic and edaphoclimatic characteristics, silvicultural management influences tree performance [38,39].

The clonal lines studied achieved the MAIs [11.1 m^3 ha^{-1} $year^{-1}$ for F24 and 83.1 m^3 ha^{-1} $year^{-1}$ for F18], and they were similar to those reported by Oliveira et al. [40] (11.03–94.99 m^3 ha^{-1} $year^{-1}$) in *E. urophylla* in Brazil. Sein and Mitlöhner [1] reported lower increments (60 m^3 ha^{-1} $year^{-1}$) in Vietnam, as did Xu et al. [46] (29.8 m^3 ha^{-1} $year^{-1}$) in China and Manasa et al. [47] (27.24–31.56 m^3 ha^{-1} $year^{-1}$) at six years of growth in India. Although the MAI responds to environmental factors, it is also influenced by age, silvicultural treatments, and the genetic characteristics of each genotype [48,49]. Therefore, different clones of the same species respond differently to environmental heterogeneity [50].

The lack of a statistically significant difference in timber volume between five and six years of age in the Fluvisol soil, and the average MAI_V of above 35 m^3 ha^{-1} yr^{-1} reported for the Huimanguillo area [4], suggests that, by selecting the five clonal lines with the best growth in each soil type, it is possible to increase plantation productivity by 50.3% to 128% in Acrisol soil, and by 11.4% to 60.28% in Fluvisol soil in the short term and to reduce the technical rotation from the currently established six years to a shorter period of five years. This could bring economic, ecological, and social benefits, such as increased profitability of the plantations due to shorter rotation periods [51]. Likewise, a smaller area would be required to produce an equivalent volume of timber, freeing up areas for other uses such as conservation and forest restoration [52]. In addition, CO_2 absorption would be favored due to a higher rate of biomass accumulation by eucalyptus trees [52,53].

The lack of statistical difference in the MAI_V between years two, three, four, five, and six in the Acrisol soil may be due to the better adaptation of the clonal lines to this soil type. Since there was mortality of the less-adapted clonal lines in all the years, the growth of the surviving trees may have compensated for the lost volume of the dead trees, which was reflected in a constant average increase in timber volume during the trial. This phenomenon was observed by Forrester et al. [54] in a trial to assess the effect of thinning on

the growth of 3.2-year-old *Eucalyptus nitens* (Deane & Maiden) in Australia. In that study, the removal of 900 to 300 trees per hectare increased the aboveground biomass growth by 34% and light use efficiency by 13% in the first year after thinning.

This could also be related to the sudden increase in CAI_V shown by some clonal lines between years five and six, although this remains unclear. Timander [55] observed a similar sudden increase in a fertilization experiment with *E. urophylla* at 4.7 years of age in China. This author attributed the response to an extra dose of phosphorus applied one year earlier. In our study, nutrient management was the same in both soil types; however, the growth response of the surviving trees in the Fluvisol shows that the properties of this soil are less favorable for *E. urophylla* growth than those of the Acrisol, since all lines presented a statistically significant reduction in MAI_V from the third year onwards, regardless of survival.

The CAI_V of 134.7 $m^3\ ha^{-1}\ yr^{-1}$ at 6 years of age observed in our study, which was considered high, was similar to that reported by Rubilar et al. [8] (10 to 100 $m^3\ ha^{-1}\ yr^{-1}$) for *Eucalyptus globulus* Labill., *E. nitens*, *Eucalyptus badjensis* Beuzev. & Welch, and *Eucalyptus smithii* F.Muell. ex R.T. Baker in Chile at 2.6 years of age. Our results are superior to those reported for *E. urophylla* by Timander [55] in China (43.25 $m^3\ ha^{-1}\ yr^{-1}$), at 5.6 years of age. In the literature, growth traits such as yield are considered to have low heritability [56,57]. In this sense, the different growth responses in terms of CAI_V are specific to each clone, as well as to the environmental conditions [57].

Clonal lines with high (>80%) survival and intermediate growth (F8, F11, F16, F19, F20, and F25) are recommended for evaluation at spacings below 1400 trees ha^{-1} (current planting density) to reduce competition. This could help to improve their productivity, although there is no recommended minimum density. Planting at below 50% of the current density (700 trees ha^{-1}) could extend the technical rotation beyond six to seven years, as it would take longer to reach maximum production per hectare [58], and could also reduce initial growth, preventing full recovery during the rotation [36].

A key strategy in forest improvement programs is to define mega-environments to assign the best genotypes to each planting area [33]. In this sense, heritability is a parameter that indicates the success of selection [59]. According to Terfa and Gurmu [60] the clonal lines showed heritabilities ranging from intermediate (0.3–0.6) to high (>0.6).

The presence of genetic variability among the clonal lines studied is evidenced by intermediate levels of H_i^2 [61]. For their part, the high values of H_c^2 suggest that the growth traits of the clonal lines studied have a strong genetic control, which also explains the lack of genotype × environment interaction. This lack of interaction could be associated with the similar temperature and precipitation conditions in the study area [15], a situation that is reflected in the positive correlation between the soil types.

The high values of H_i^2, H_c^2, and r_{gg} [61] suggest a favorable situation for the identification of clonal lines with high genetic potential for timber production in both soil types [62,63]. These values were higher than the heritabilities estimated for *E. urophylla* clonal lines (0.38 to 0.48) in southern China [64] and Brazil (0.54–0.58) [62]; however, heritabilities of 0.94 to 0.99 in diameter, height, and volume per hectare have also been reported in China for hybrid clonal lines with *E. urophylla* as one of the parents [63].

The Cv_e values of timber volume per hectare are considered medium (31.75%) to high (39.63–42.48 %) [65]. These values are common in field trials due to the difficulty in controlling the effects of the environment [66]; in volume per hectare, they are considered expected values since this trait is a result of diameter, height, and survival; thus, it accumulates the environmental variation in these characteristics [67]. Our values were similar to those of clonal trials of *Eucalyptus* in South America [25,67,68].

The positive correlations observed indicate a strong relationship between the growth variables, which is common in forestry studies. High genetic correlations have been observed in other studies of *Eucalyptus* spp. [62,64,69], *Populus* spp. hybrid clonal lines [70], and *P. taeda* [23], suggesting that growth characteristics are regulated by genes with a pleiotropic effect [30]. The higher correlations between diameter and volume coincide with

those found in the studies mentioned above, indicating that selecting genotypes by diameter would imply selecting the tallest trees with the highest volume per hectare in this group of clonal lines, and diameter is easier to measure than tree height. Some studies report genetic correlations in excess of 0.90 between height and volume [30,63], values that are higher than those found in our study.

In Figure 4d (which-won-where), clonal lines that do not fall within a given soil type are considered unfavorable for the soil and spacings tested [71]; however, the number of environments formed indicates the genetic potential of the clonal lines for testing in other soil and climatic conditions [72].

The generation of a single mega-environment is beneficial for the breeding program of *E. urophylla* in the Huimanguillo region since two or more breeding environments may hinder operations and raise the costs of production, handling, and the transport of the plant to the different planting areas.

Although the Fluvisol soil presents better nutrient and organic matter conditions for the growth of plant species compared to the Acrisol soil [14], it has been observed that the productivity of *E. urophylla* is not exclusively linked to fertility. Previous studies have found that *Eucalyptus* spp. has a high nutrient use efficiency [73,74], such that nutrient status is not usually the main limiting factor in the development of *Eucalyptus* plantations [75]. Delgado-Caballero et al. [5] and Pérez-Sandoval et al. [6] reported that soil texture in southeastern Mexico, with values of 44% clay and 30% sand, presented the most productive site indices, regardless of fertility. This could be because texture influences soil water content [76]. Stape et al. [75] and Otto et al. [77] demonstrated the sensitivity of *Eucalyptus* spp. to water availability, finding a greater positive response of trees to water supply than to fertilization.

The diverse growth and mortality responses of the clonal lines of *E. urophylla* corroborate the high genetic diversity that occurs naturally in the species [78,79]. This confirms that, despite the progress made, breeding programs for *E. urophylla* in southeastern Mexico are still in their early stages. In an environment of climate change, it is necessary to continue evaluating genotypes to maintain a broad genetic base, complemented by soil studies, to develop new genetic improvement strategies for the species and thus maintain the productivity of clonal lines without deteriorating the productive capacity of these soils. This is the only way to achieve sustainable timber production for the MDF and sawmill industry in the coming years.

5. Recommendations

The use of the clonal lines F5, F7, F23, F21, F18 in the Acrisol soil and the clonal lines F23, F13, F21, F2, F18 in the Fluvisol soil is recommended. Moreover, we recommend maintaining the other 21 clonal lines in reserve in order to preserve a broad genetic base as a security measure in case the recommended clonal lines fail to adapt.

6. Conclusions

Growth and survival were controlled by the genetic constitution of the clonal lines and the particular conditions of each soil type. The growth of the clonal lines was found to be stable since no genotype × environment interaction effects were observed. The greatest tree dimensions were observed in the Acrisol soil.

The observed high productivity levels and the high heritability of the growth traits in the clonal lines indicate the potential to use these lines to increase the productivity of the plantations in Huimanguillo by 11.4% up to 128% and to reduce the technical rotation from six to five years.

Due to the high precision of selection and the significant positive genetic correlations observed between the diameter, height, and volume per hectare, it is feasible to select productive clonal lines using diameter as a selection criterion. This is important since it would allow optimization of measurement and selection fieldwork, given that diameter is an easily measurable variable.

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