

Clonal selection and genotype-by-environment interaction in *Eucalyptus* for sustainable forestry in Alagoas

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SILVICULTURE

ABSTRACT

Background: The expansion of clonal eucalypts forestry into new forest frontiers in Northeastern Brazil requires selecting genotypes adapted to local conditions, thereby reducing pressure on native biomes such as the Caatinga. This study evaluated genotype-by-environment (G×E) interaction across four sites in Alagoas State based on mean annual increment (MAI) and survival (SUR) using 40 clones. The REML/BLUP, HMRPGV, and GGE biplot methodologies were applied, and following the analyses, an empirical index combining MAI and survival rate was employed for clone selection.

Results: A significant G×E interaction was detected, enabling the identification of two breeding zones and two mega-environments. Clones with high productive potential were selected. The HMRPGV method resulted in the highest genetic gain, while adjusting MAI by survival rate reshaped clone ranking and selection outcomes.

Conclusions: These findings provide robust recommendations for Alagoas, fostering efficient land use, reducing pressure on the Caatinga biome, and supporting the sustainable expansion of the regional forest base.

Keywords: Forest improvement; GGE biplot; REML/BLUP; Silviculture.

HIGHLIGHTS

G×E analysis defined two breeding zones to guide regional clone deployment.
Superior clones were identified to enhance timber supply in Alagoas.
MAI correction by survival improved clone selection.
HMRPGV increases genetic gain and strengthens sustainable forest development.

NUNES, T. S.; ARRIEL, D. A. A.; ALEXANDRE, F. S.; ALFENAS, R. F.; GUIMARÃES, L. M. S.; MACEDO, D. M.; ALFENAS, A. C. Clonal selection and genotype-by-environment interaction in *EUCALYPTUS* for sustainable forestry in Alagoas. CERNE, v. 32, e103647, 2026. DOI: 10.1590/01047760202632013647

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Scientific Editor: Evandro Vagner Tambarussi

Received: October, 04 2025
Accepted: April 17, 2026

INTRODUCTION

Eucalyptus spp. is the most extensively planted forest genus in Brazil, with an average productivity of 34.4 m³ ha⁻¹ year⁻¹ (IBA, 2025). The country is globally recognized for the high productivity of its eucalypts plantations (Silva *et al.*, 2025). However, in recent years, productivity gains have stagnated due to a combination of factors, including the expansion of plantations into non-traditional regions, climate variability, suboptimal management practices, the emergence of new pests and diseases and limited knowledge regarding optimal clone-site matching (Cunha *et al.*, 2021). Forest breeding programs focused on selecting well-adapted genotypes play a crucial role in addressing these challenges (Florêncio *et al.*, 2022). In this context, clonal testing stands out as a key stage (Oliveira *et al.*, 2023), enabling faster and more precise identification of superior genetic material, thereby enhancing both economic performance and ecological sustainability.

The need to select high-yielding eucalypts clones is particularly urgent in certain states of Northeastern Brazil, where intense demand for firewood and charcoal places substantial pressure on the native Caatinga biome. When supported by appropriate management practices and environmentally sound site-zone matching, eucalypts cultivation can serve as a sustainable alternative that helps alleviate this pressure (Sembiring *et al.*, 2025). In Alagoas state, where eucalypts cultivation is still in its early stages (Morais *et al.*, 2017), the species presents a promising alternative for mitigating environmental degradation. Preliminary estimates suggest productivity levels of up to 45 m³ ha⁻¹ year⁻¹ during the first rotation cycle (6–7 years) (Angeiras, 2016; Floriano, 2018). However, despite this potential, no prior studies have been conducted to identify clones best adapted to the region's specific edaphoclimatic conditions and the magnitude of G×E interaction. This gap in knowledge limits the ability to delineate breeding zones and to recommend the most suitable genetic materials for the region.

A major challenge in identifying superior genetic material is the genotype-by-environment (G×E) interaction (Costa *et al.*, 2024). As eucalypts cultivation advances to new frontiers in the country, the G × E interaction becomes a significant challenge for the safe recommendation of clones in these new environments (Reis *et al.*, 2025). When this interaction is complex, it necessitates the establishment of specific “breeding zones” with tailored improvement programs. G×E interaction is pervasive in forest genetics (Callister *et al.*, 2024) and many statistical approaches are available to assess it, such as the harmonic mean of the relative performance of genotypic values (HMRPGV) that integrates stability, adaptability, and productivity into a unified statistic for the selection of superior genetic materials (Resende, 2007); and the GGE (G, genotype + GE, genotype-by-environment interaction) biplot method, employing multivariate analysis, enables visualizing results graphically, facilitating the identification of pertinent information (Yan, 2001).

It is hypothesized that a significant genotype-by-environment interaction exists among *Eucalyptus* clones evaluated under different edaphoclimatic conditions in Alagoas, enabling the identification of distinct breeding zones and the selection of superior genotypes for productivity and survival.

Given the urgent need to select productive *Eucalyptus* clones for new Brazilian forest frontiers, the general objective of this study was to select *Eucalyptus* clones adapted to the conditions of Alagoas State. Specifically, the study aimed to: (i) evaluate G×E interaction using REML/BLUP, HMRPGV, and GGE biplot methodologies; (ii) define breeding zones based on genotypic correlations among environments; (iii) estimate genetic gains considering the adjustment of mean annual increment (MAI) by survival.

MATERIALS AND METHODS

Location and Characterization of the Experimental Sites

The clonal trials were established in 2009 across four municipalities in the state of Alagoas, Brazil: Cajueiro (Usina Capricho – UO), Maceió (Usina Cachoeira – UA), Passo de Camaragibe (Santa Justina Farm – SJ), and Penedo (Usina Marituba – UM) (Figure 1). The test sites were selected based on a study conducted prior to the installation of the experiments (unpublished data), which used the Physiological Processes Predicting Growth (3-PG) model (Landsberg and Waring 1997) to estimate the potential productivity of eucalypts in Alagoas, integrating long-term climate and soil attributes. From these, locations were chosen that represent the main climate–soil productivity classes for the species in the state, providing a wide yet realistic gradient of site quality for evaluating clonal performance and genotype-by-environment (G×E) interactions. The experimental sites differed in their climatic characteristics, including minimum and maximum temperatures, humidity, and rainfall (Figure 1 and Table 1).

Experimental Design and Genetic Material

The study was conducted using a randomized complete block design with six replicates. Each experimental plot consisted of nine plants (three rows × three plants), with a spacing of 2.5 × 2.5 m, covering a total area of 6.25 m² per plant. The total area for each experiment was 2 hectares.

The clonal trials included a total of 40 *Eucalyptus* clones. The experiments at Santa Justina Farm (SJ) and Usina Capricho (UO) utilized all 40 clones. The trials at Usina Marituba (UM) and Usina Cachoeira (UA) used the same genotypes, except for clones CLR 1032 and CLR 1034 (Table 2).

Cultural practices and plantation

Prior to planting, the areas were cleared, and leaf-cutting ants were controlled. Planting was carried out during

Table 1: Edaphoclimatic conditions of the sites where clonal trials were installed in the state of Alagoas, Brazil.

	Santa Justina Farm	Usina Cachoeira	Usina Capricho	Usina Marituba
Municipality	Passo de Camaragibe	Maceió	Cajueiro	Penedo
Latitude (S)	-09° 12' 18 6"	-09° 25' 23 8"	-09° 21' 24 9"	-10° 12' 59 2"
Longitude (W)	-35° 30' 54 24"	-35° 43' 57 3"	-36° 08' 36 0"	-36° 28' 27 6"
Annual precipitation (mm)	1.600	1.250-1.500	1.250-1.500	1.000-1.250
Altitude (m)	16	96	141	84
Minimum temperature (°C)	21.5	22.2	20.5	21.5
Maximum temperature (°C)	31	29,9	31	31
Relative humidity (%)	82	81	75	60
Biome	Atlantic forest	Atlantic forest	Atlantic forest	Atlantic forest
Köppen climate classification	Humid	Humid	Humid subhumid	Dry subhumid
Solo Type	Neossolo	Oxisol Yellow	Yellow clayey	Yellow clayey
Texture	Clayey	Medium clay	Medium sandy	Medium sandy

the regional rainy season (July to August). Basal fertilization was applied according to soil analysis recommendations for each specific site. Replanting was performed 25 days after initial planting to ensure a full stand.

RESULTS

Data collection

At 66 months of age (5.5 years), the following assessments were performed on all plants: stem form, incidence of diseases, survival rate (SUR), circumference at breast height (CBH, measured at 1.30 m above ground), and total height (H, in meters). CBH was measured with a measuring tape and later converted to diameter at breast height (DBH). Height was measured using a digital hypsometer. Tree volume was estimated using the cylindrical equation, adjusted with a form factor of 0.5. These values were then scaled to a per-hectare basis and divided by stand age to calculate the Mean Annual Increment (MAI, expressed in $\text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$). All analyses were based on individual tree measurements rather than plot averages, thereby enhancing the accuracy of the estimates.

G×E Interaction Analysis (REML/BLUP)

The analysis of the genotype-by-environment (G×E) interaction for MAI and SUR was performed using

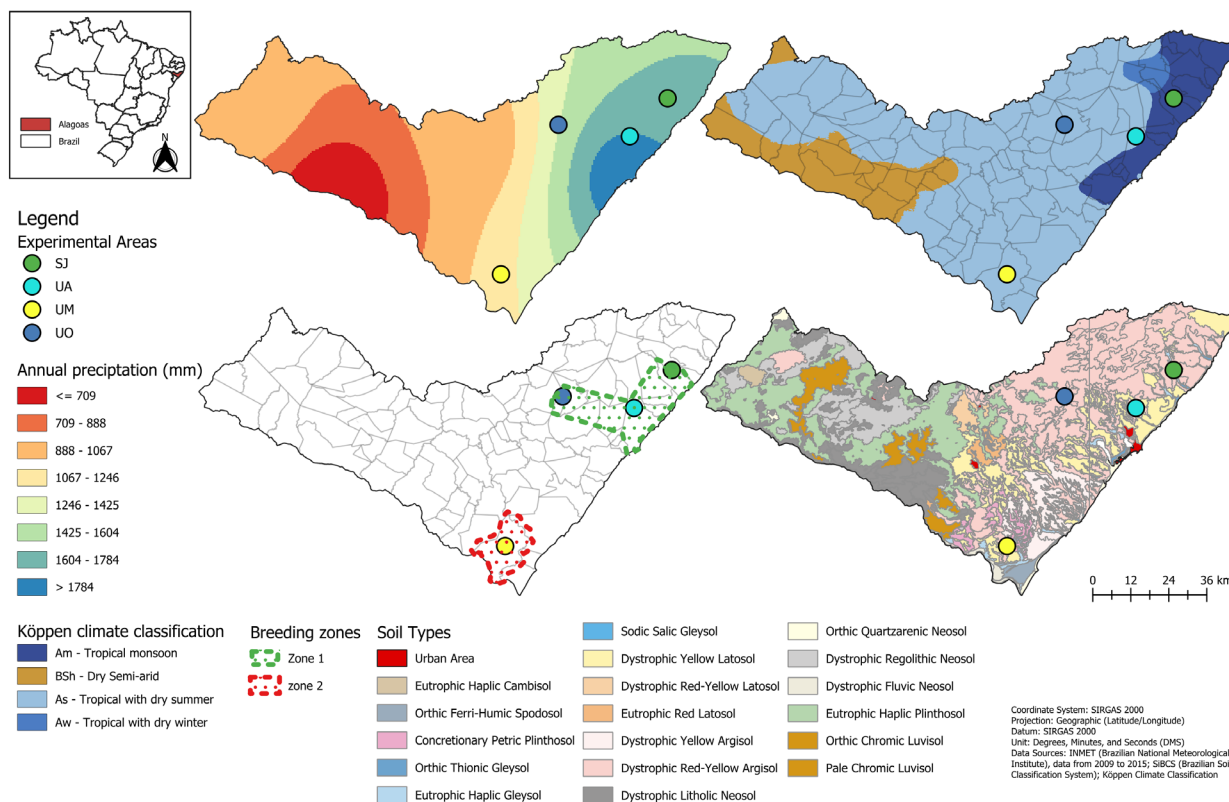


Figure 1: Experimental areas of eucalypts clonal trials in Alagoas, Brazil: Passo de Camaragibe (Santa Justina- SJ), Maceió (Usina Cachoeira- UA), Cajueiro (Usina Capricho- UO), and Penedo (Usina Marituba- UM). A: Annual precipitation B. Köppen climate classification; C: Breeding zones for MAI (Mean Annual Increment, $\text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$); D: Soil types.

Table 2: *Eucalyptus* clones used in clonal trials in Alagoas, Brazil.

Clones	Species/hybrid	Clones	Species/hybrid
CLR 1000	<i>E. urophylla</i> × <i>E. camaldulensis</i>	CLR 1020	<i>E. grandis</i>
CLR 1001	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1021	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1002	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1022	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1003	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1023	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1004	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1024	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1005	<i>E. urophylla</i> × <i>E. grandis</i>	CLR 1025	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1006	<i>E. grandis</i>	CLR 1026	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1007	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1027	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1008	<i>E. grandis</i>	CLR 1028	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1009	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1029	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1010	<i>E. urophylla</i> × <i>E. grandis</i>	CLR 1030	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1011	<i>E. grandis</i>	CLR 1031	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1012	<i>E. urophylla</i> × <i>E. grandis</i>	CLR 1032*	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1013	<i>E. urophylla</i>	CLR 1033	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1014	<i>E. urophylla</i> × <i>E. grandis</i>	CLR 1034**	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1015	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1035	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1016	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1036	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1017	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1037	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1018	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1038	<i>E. grandis</i> × <i>E. urophylla</i>
CLR 1019	<i>E. grandis</i> × <i>E. urophylla</i>	CLR 1039	<i>E. urophylla</i> × <i>E. camaldulensis</i>

* Clone not used at Usina Marituba; ** Clone not used at Usina Cachoeira

the restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) approach via Model 51 of the SELEGEN-REML/BLUP software (Resende, 2016). The model is described as (Equation 1):

$$y = Xr + Zg + Wp + Ti + e \quad (1)$$

Where: y is the vector of data; r is the vector of fixed block effects (plus the overall mean); g is the vector of random genotypic effects; p is the vector of random plot effects; i is the vector of random $G \times E$ interaction effects; e is the vector of random residuals; X , Z , W , T are the incidence matrices for the respective effects.

The random effects follow the distributions:

$$g \sim N(0, I\sigma_g^2), p \sim N(0, I\sigma_p^2), i \sim N(0, I\sigma_i^2), e \sim N(0, I\sigma_e^2).$$

Thus, the variance-covariance structure of the observation vector is:

$$\text{Var}(y) = Z_g G Z_g' + Z_p B Z_p' + Z_i I \sigma_i^2 + R,$$

with:

$$G = I\sigma_g^2, B = I\sigma_p^2, GL = I\sigma_i^2, R = I\sigma_e^2.$$

Therefore:

$$\text{Var}(y) = Z_g I\sigma_g^2 Z_g' + Z_p I\sigma_p^2 Z_p' + Z_i I\sigma_i^2 Z_i' + I\sigma_e^2.$$

The significance of the random effects was assessed using the likelihood ratio test (LRT) at a 1% significance level within the analysis of deviance (ANADDEV). Variance components and genetic parameters were estimated via REML. Pairwise analyses between environments were conducted to identify breeding zones and optimize the selection strategy.

Selection of Superior Genetic Material

The selection of the best-performing clones was conducted using the following strategies:

Direct Selection for MAI: for joint and individual analysis of environments.

Selection for Stability and Adaptability: The harmonic mean of the genotypic values (HMGV) method was employed. This procedure, based on the BLUP of genotypic values, provides three key parameters:

- HMGV: Harmonic mean of genotypic values (a measure of overall productivity penalized by instability).

- RPGV: Relative performance of genotypic values (adaptability per environment).

- HMRPGV: Harmonic mean of the relative performance of genotypic values (a combined measure of mean performance, stability, and adaptability). The top 10 clones were selected based on the HMRPGV values.

Following the analyses, an empirical index combining MAI and SUR was adopted. This index was employed to adjust MAI according to SUR performance, thereby avoiding the overestimation of clones with high productivity but low survival. The formula (2) used was

$$IE = MAI \times S \quad (2)$$

where IE represents the empirical index, MAI is the mean annual increment ($m^3 \text{ ha}^{-1} \text{ year}^{-1}$), and S is the survival rate expressed as a proportion.

This practical index is widely applied in field evaluations because it provides a straightforward integration of two essential traits for plantation success: growth and individual persistence. Its use is particularly relevant in tree breeding programs, as it ensures that selection decisions consider both productivity and survival, contributing to the long-term sustainability and economic viability of forest plantations.

GGE Biplot Analysis

For a graphical assessment of the $G \times E$ interaction for MAI, a GGE Biplot analysis was performed based on the model proposed by Yan *et al.* (2001), as follow (equation 3):

$$Y_{ij} = \mu + G_i + E_j + GE_{ij} + e_{ij} \quad (3)$$

Where: Y_{ij} is the mean performance of genotype i in environment j , μ is the overall mean, G_i is the effect of genotype i , E_j is the effect of environment j , GE_{ij} is the interaction effect, and e_{ij} is the error term.

The following biplot interpretations were used: "Which-won-where" to identify mega-environments and winning genotypes; "Ideal genotype" to identify clones with high performance and stability; "Discriminateness vs. representativeness" to assess the environments; and "Ideal environment" to identify the most representative and discriminative test sites.

RESULTS

Variance Components and G×E Interaction

The effects of clones, plants within plots, and their interaction were significant for both MAI and SUR (Table 3). This significance indicates that the behavior of the clones is not uniform or proportional to environmental changes. The coefficient of determination for the G×E interaction effects (C^2_{int}) was 0.03 for MAI, suggesting a moderate genotypic correlation between the environments (Table 4). For survival, the C^2_{int} was 0.16, indicating a stronger environmental influence on clone performance (Table 4). The genotypic correlation across environments (r_{gloc}) was moderate for MAI (0.54) and low for SUR (0.37). These r_{gloc} values confirm that the G×E interaction is of the complex type, meaning a general recommendation of clones for all environments is not advised. The heritability of the mean for clones (h^2_{mc}) was high for both traits, with values of 0.68 for MAI and 0.65 for SUR. The accuracy of clone selection (A_{cgen}) was also high, with values greater than 0.80 for both MAI and SUR. The coefficient of variation for residuals (CV_r) for MAI was 0.41, while for SUR it was 0.64, suggesting that MAI was more influenced by the environment than survival (Table 4).

Table 3: Analysis of deviance for mean annual increment ($m^3\ ha^{-1}\ year^{-1}$) and survival of Eucalyptus clones in clonal trials in the state of Alagoas, Brazil, at 66 months.

Effect	Mean Annual Increment (MAI)		Survival	
	Deviance.	LRT	Deviance.	LRT
Clones	48333.92	17.58**	-9661.49	18.61**
Plot	48643.61	327.27**	-8976.49	703.61**
Interaction	48331.76	15.42**	-9480	200.1**
Full model	48316.34		-9680.1	

Dev. = Deviance; LRT = maximum likelihood ratio test. ** significant at 1% probability with 1 degree of freedom.

Breeding zones

The genotypic correlation of clone performance for MAI between the paired locations was as follows: Santa Justina × Usina Cachoeira: 0.81; Usina Cachoeira × Usina Capricho: 0.73 and Santa Justina × Usina Capricho: 0.69 (Table 5). A joint analysis of these three environments resulted in a r_{gloc} of 0.74. Usina Marituba, however, showed a low correlation (below 0.70) with all other sites. Based on these results, two distinct breeding zones were established: the first comprising Santa Justina, Usina Cachoeira, and Usina Capricho, and the second consisting solely of Usina Marituba. For the survival trait, all environments' pairs exhibited low correlations, which suggests a complex-type interaction and makes it unfeasible to create breeding zones based on this characteristic.

Table 4: Estimates of variance components and genetic parameters for mean annual increment ($m^3\ ha^{-1}\ year^{-1}$) and survival characters in 66-month-old eucalypts clonal trials in Alagoas, Brazil.

Parameter	Mean Annual Increment	Survival
V _g	43.07	0.01
V _{par}	170.83	0.03
V _{int}	36.38	0.03
V _e	824.13	0.10
V _f	1074.41	0.16
h _{2g}	0.04	0.09
C _{2par}	0.16	0.16
C _{2int}	0.03	0.16
h _{2mc}	0.68	0.65
A _{cgen}	0.82	0.81
r _{gloc}	0.54	0.37
CV _{gi} (%)	10.27	17.01
CV _e (%)	25.35	26.54
CV _r	0.41	0.64
Mean	63.90	0.72

V_g: genotypic variance among genotypes (clones); V_{par}: variance between plots; V_{int}: variance of G×E interaction; V_e: residual variance; V_f: individual phenotypic variance; h_{2g}: individual heritability in the broad sense of individual plots in the block; C_{2par}: coefficient of determination of plot effects; C_{2int}: coefficient of determination of the effects of the G×E interaction; h_{2mc}: heritability of mean of clones; A_{cgen}: accuracy of clone selection; r_{gloc}: genotypic correlation between clone performance in different environments; CV_{gi}: genotypic variation coefficient; CV_e: residual coefficient of variation; CV_r: coefficient of relative variation.

Table 5: Genetic correlation between environments evaluated in pairs for mean annual increment ($m^3\ ha^{-1}\ year^{-1}$, upper diagonal) and survival (lower diagonal), at 66 months for *Eucalyptus* clones in clonal trials in Alagoas, Brazil.

Sites	Mean Annual Increment (MAI)			
	Santa Justina	Usina Cachoeira	Usina Marituba	Usina Capricho
Santa Justina	-	0.81	0.14	0.69
Usina Cachoeira	0.44	-	0.62	0.73
Usina Marituba	0.25	0.31	-	0.34
Usina Capricho	0.54	0.20	0.57	-

Selection and estimates of genotypic values in the joint analysis of environments

The ranking of the top 10 clones for MAI changed after the productivity values were corrected for survival. This change occurred because clones with high survival advanced in the ranking, while those with high mortality fell in position. The MAI mean, adjusted for survival for all 40 clones, was $46.2\ m^3\ ha^{-1}\ year^{-1}$. With the selection of the top 10 clones, the genetic gain was 10.26% (Figure 2).

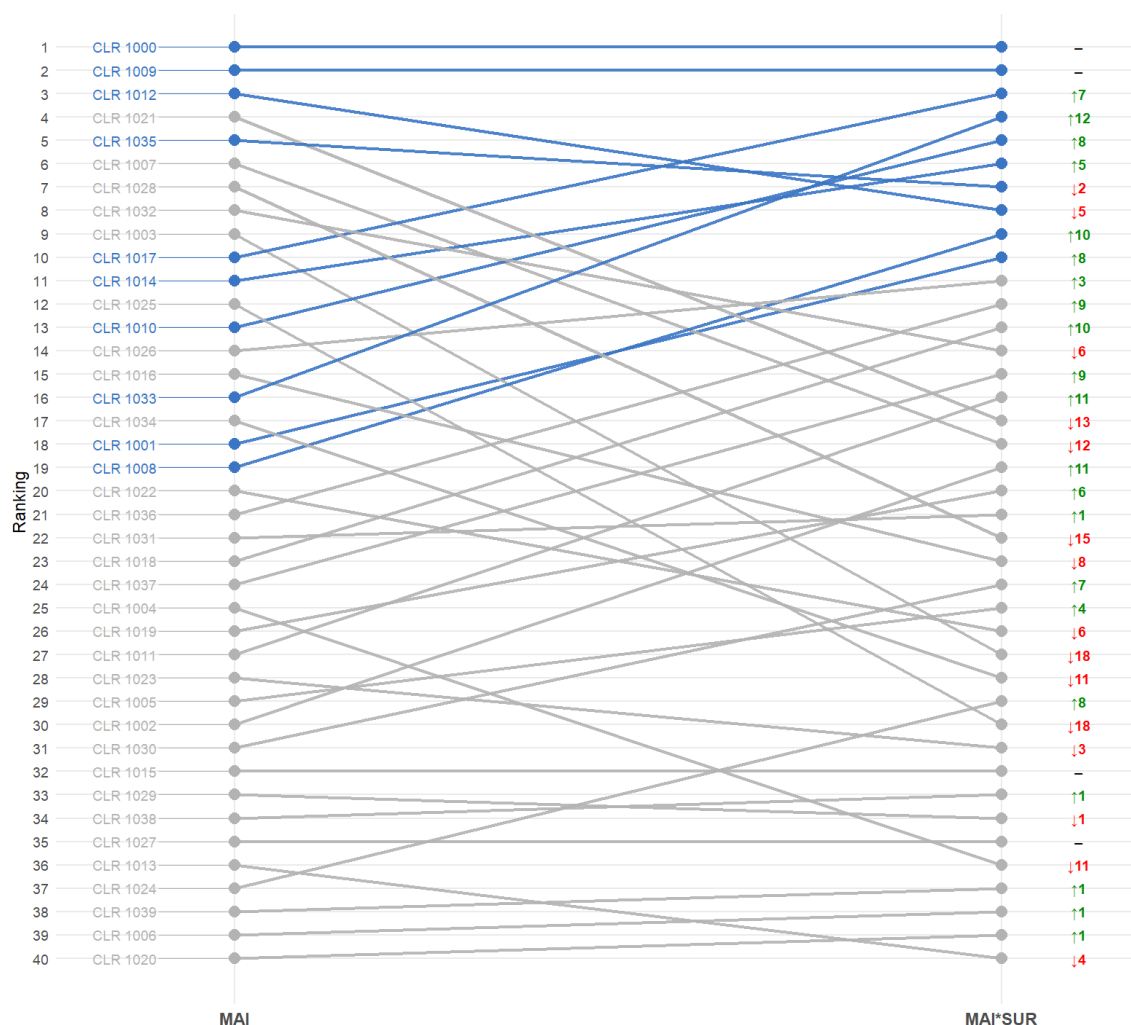


Figure 2: Ranking of *Eucalyptus* clones for mean annual increment (MAI, $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) at 66 months, based on the joint analysis of environments in the state of Alagoas, Brazil. The left panel presents the ranking derived from the original MAI genotypic values, while the right panel shows the ranking of MAI values adjusted by the mean survival rate (SUR) of each clone. Lines connect the position of each clone between panels, highlighting changes in ranking when survival is incorporated. The selection gain for the top 10 clones was 6.53% using MAI alone and 10.26% when MAI was multiplied by SUR.

Selection and estimation of genotypic values in the analysis of individual environments

The MAI for Usina Capricho, Santa Justina, Usina Marituba and Usina Cachoeira were 65.9, 70.2, 59.3 and 60, respectively. The experimental areas with the highest survival rates at 66 months were Usina Capricho (91%) and Santa Justina (83%). Usina Marituba and Usina Cachoeira exhibited the lowest survival rates, with 68% and 46%, respectively. The mean MAI, multiplied by survival, was 58.3, 27.6, 60.0, and $40.3 \text{ m}^3 \text{ha}^{-1} \text{year}^{-1}$ for Santa Justina, Usina Cachoeira, Usina Capricho, and Usina Marituba, respectively. In this case, the correction of the individual genotypic values for MAI was achieved by multiplying the survival rates of the clones in each environment. The multiplication of the MAI for survival not only changed the productivity by location and

the ranking of the most productive sites, but also altered the clones selected for each location.

Individual selection per environment (Figure 3) resulted in greater genetic gains than joint selection across all sites (Figure 2). As with the joint analysis, when MAI was multiplied by SUR, there were changes in the ranking of the best clones across all environments (Figures 3A and 3B). For Usina Cachoeira and Usina Marituba, only 40% of the clones were the same, while for Santa Justina the correspondence was 70%, and for Usina Capricho it was 80%, with some position reversals among the clones (Figures 3A and 3B).

The mean MAI of the 10 best clones, multiplied by survival, was 74.5 (gain of 16.18%), 72.5 (gain of 12.54%), 49.7 (gain of 22.08%), and $54.8 \text{ m}^3 \text{ha}^{-1} \text{year}^{-1}$ for Santa Justina, Usina Capricho, Usina Cachoeira, and Usina Marituba, respectively (Figure 3).

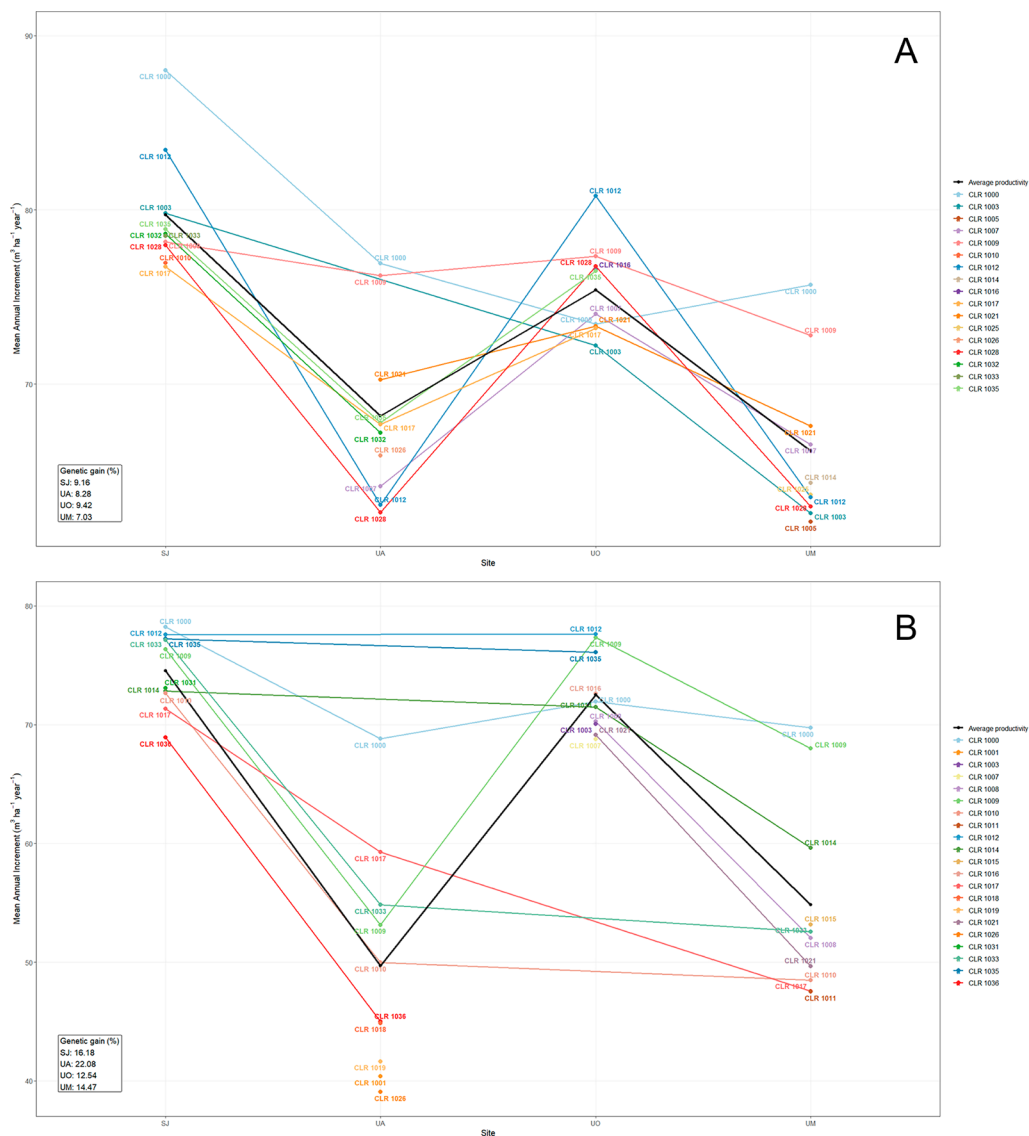


Figure 3: BLUP estimate for mean annual increment (MAI) and genetic gain for the 10 best *Eucalyptus* clones of the trials in the state of Alagoas, Brazil, at 66 months of age. MAI not multiplied for survival (A) and MAI multiplied for survival (B). The black line indicates the average productivity of the site. SJ = Santa Justina, UA = Usina Cachoeira, UO = Usina Capricho, UM = Usina Marituba.

Selection for stability, adaptability, and productivity

The analysis of the HMGV, RPGV, and HMRPGV methods revealed that the top 10 clones were nearly identical across the three parameters. CLR 1036 was the only clone that appeared solely in the stability analysis, not ranking among the top 10 for RPGV and HMRPGV (Figure 4). For the top 10 clones, the mean MAI multiplied by survival was 58.0 (gain of 45.90%), 62.2 (gain of 34.25%), and 60.4 (gain of 41.38%) $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ for HMGV, RPGV, and HMRPGV, respectively. CLR 1000 and CLR 1009 stood out, ranking first and second in all three analyses, indicating they possess high productivity, stability, and adaptability (Figure 4).

GGE biplot

The first two principal components explained 80.5% of the MAI variation (without correction for survival). Clone CLR 1009 can be considered the closest to the "ideal genotype" (represented by the concentric circle with an arrow in Figure 5A), meaning it has relatively higher productivity across all environments. This clone also ranks among the best in stability, adaptability, and productivity analyses. Two mega-environments were identified: the first, formed by Usina Capricho and Usina Marituba, where Clone CLR 1012 was the best performer, and the second, formed by Santa Justina and Usina Cachoeira, with clones CLR 1000, CLR 1033, and CLR 1009 exhibiting the best performance (Figure 5B).

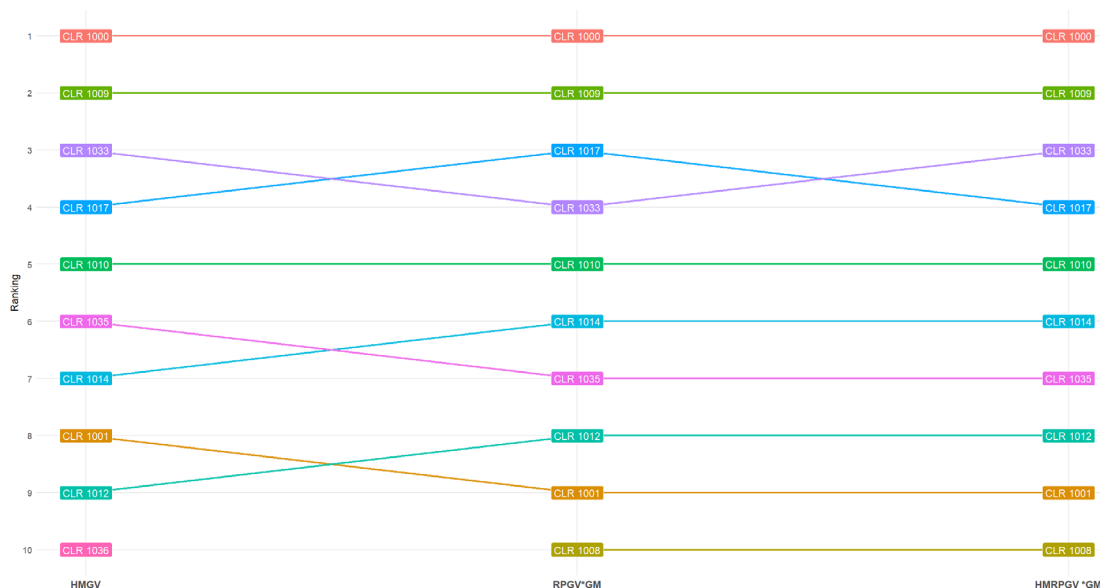


Figure 4: Ranking of Eucalyptus clones based on stability (HMGV), adaptability (RPGV and RPGV*GM), and the combined index of stability, adaptability, and productivity (HMRPGV and HMRPGV*GM) for mean annual increment (MAI, $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) multiplied by survival in the joint analysis of environments at 66 months in the state of Alagoas, Brazil. Lines connect the ranking positions of each clone across the different simultaneous selection indexes, highlighting shifts in performance depending on the criterion applied. The estimated genetic gains were 45.90%, 34.25%, and 41.38% in MAI $\times$ survival for HMGV, RPGV, and HMRPGV, respectively.

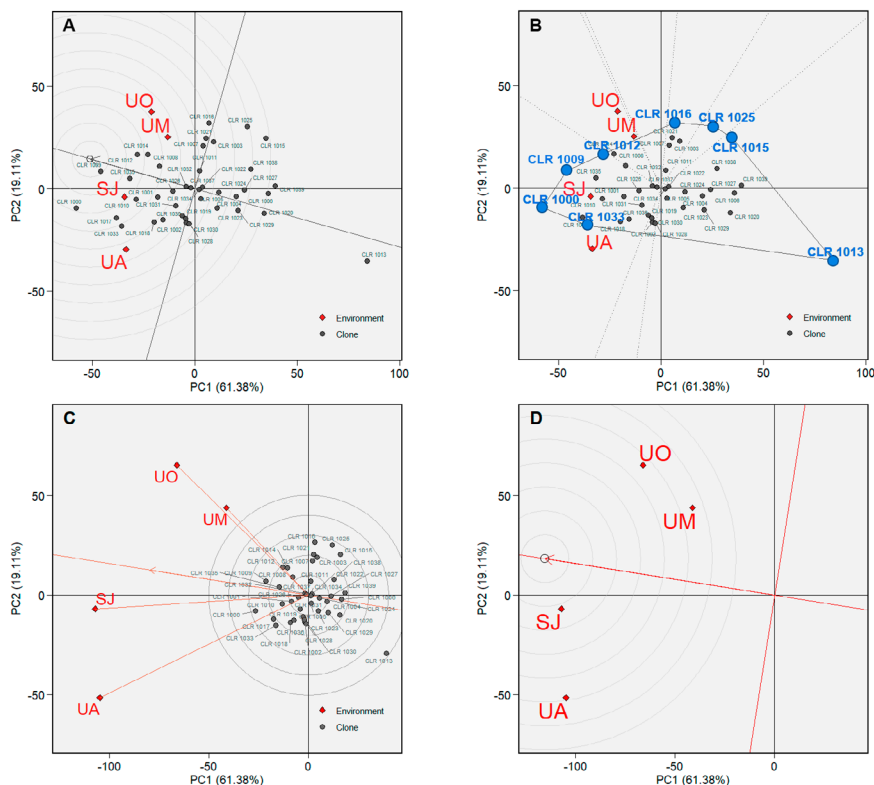


Figure 5: GGE biplot: ideal clone (A); mega-environments (which-won-where) (B); discrimination and representativeness environment (C) and the ideal environment (D) for the experimental network in the state of Alagoas, Brazil, for the mean annual increment (MAI, $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) of Eucalyptus clones at 66 months. SJ = Santa Justina; UA = Usina Cachoeira; UO = Usina Capricho; and UM = Usina Marituba

Each mega-environment represents locations where the most productive clones were the same, and genotypes located in groups that do not contain environments are considered unfavorable for the environments in which they were tested.

The discrimination and representativeness graph indicates the ideal location for selecting the most productive clones in the experimental network and for identifying the most representative site of the network, respectively (Figure 5C). Usina Cachoeira is the most discriminative among the sites, as indicated by the longest vector, while Fazenda Santa Justina (SJ) is the most representative site, being closest to the midline (Figure 5C). Among the evaluated sites, Usina Marituba is the least discriminating and least representative. The closer a test environment is to the 'ideal' environment, the greater its discrimination and representativeness. Therefore, among the sites evaluated, Fazenda Santa Justina (SJ) demonstrates strong representation and discrimination and should be prioritized for the establishment of new trials and the selection of the best genotypes, as it is closest to the central concentric circle (Figure 5D).

DISCUSSION

This study identified *Eucalyptus* clones with high productive potential for the edaphoclimatic conditions of Alagoas and demonstrated that genotype-by-environment (G×E) interaction is complex for both mean annual increment (MAI) and survival (SUR), as indicated by moderate to low genotypic correlations across environments ($r_{\text{gloc}} = 0.54$ for MAI; 0.37 for SUR). Two breeding zones were delineated—Zone 1 (Santa Justina, Usina Cachoeira, and Usina Capricho) and Zone 2 (Usina Marituba)—reflecting distinct adaptation patterns. Adjusting MAI by SUR reshaped clone rankings and improved the robustness of recommendations, while HMRPGV provided the highest selection gain among the simultaneous selection indices.

Genotypes and the G×E interaction were modeled as random effects within the REML/BLUP framework to predict breeding values rather than merely compare treatment means. BLUP produces shrinkage estimates that mitigate environmental noise and unbalanced data, yielding unbiased predictions of genotypic values and higher selection accuracy (Resende 2007; 2016). The accuracy of clone selection (A_{gen}) and the heritability of the mean for clones (h^2_{mc}) was high (Resende and Duarte, 2007) for both traits, indicating a reliable selection process and considerable genetic control over the studied traits. Even though the CV_r ratio values were low (less than 1), indicating greater environmental variation for the MAI and SUR traits, it was still possible to achieve genetic gains through selection. It was expected that lower values of C^2_{int} would correspond to higher estimates of r_{gloc} . However, this pattern was not observed for MAI in this study. The inconsistency found among these genetic parameters may be related to the influence of genotype-by-environment (G×E) interaction and unpredictable climatic factors on clone performance,

which may explain the moderate genotypic correlation (0.54) observed for MAI.

The complex G×E pattern is consistent with the strong environmental gradient among test sites in Alagoas. The cluster formed by Santa Justina, Usina Cachoeira, and Usina Capricho displayed higher genotypic correlations for MAI (0.69–0.81), supporting a shared adaptation domain (Zone 1), whereas Usina Marituba showed lower correlations with all other sites, behaving as a distinct adaptation domain (Zone 2). These patterns likely reflect differences in rainfall regime, relative humidity, and soil texture/structure documented for the sites (Figure 1; Table 1), which influence water balance, nutrient availability, and stress tolerance—key drivers of clone performance. A similar pattern has been observed in other clonal trials (Oliveira *et al.*, 2020; Jatzek *et al.*, 2022; Reis *et al.*, 2025), as well as in progenies tests (Munhoz *et al.*, 2021; Silva *et al.*, 2022) carried out in Brazil.

The GGE biplot corroborated these domains by identifying two mega-environments and highlighting winning genotypes within each; for example, CLR 1012 in the Capricho–Marituba group, and CLR 1000/1033/1009 in the Santa Justina–Cachoeira group, with CLR 1009 close to the 'ideal genotype' configuration. Furthermore, Santa Justina emerged as the most representative site of the network and Usina Cachoeira as the most discriminating, providing complementary roles for future testing and selection (Figure 5). Santa Justina exhibited the best balance between representativeness and discriminativeness, thus emerging as the ideal environment within the testing network. This outcome is consistent with its intermediate edaphoclimatic attributes (Figure 1; Table 1), which mirror conditions commonly found in areas with strong potential for forest expansion in Alagoas. Consequently, Santa Justina represents the most suitable site for conducting new clonal trials and for validating the genetic materials selected for regional deployment.

Given the delineation of two breeding zones, we recommend deploying multiple clones within each zone to balance genetic gain and reduce vulnerability to pests, diseases, or climatic extremes—risks that tend to disproportionately affect single-clone deployments, especially under complex G×E interactions (Rezende *et al.*, 2019; Oliveira *et al.*, 2023). In Zone 1 (Santa Justina–Cachoeira–Capricho), the elite set should prioritize clones that consistently ranked among the best in stability/adaptability/productivity (e.g., CLR 1000 and CLR 1009), while leveraging the representativeness of Santa Justina and the discriminative power of Cachoeira for ongoing selection. In Zone 2 (Marituba), the deployment set should center on the best performers identified for that environment (e.g., CLR 1012 and CLR 1001) and be validated under local operational conditions. The incorporation of the empirical index ($IE = MAI \times S$) into the selection process significantly influenced clone ranking compared to analyses based solely on MAI. While clones with high growth potential were initially prioritized, adjusting MAI by survival rate highlighted genotypes that combined productivity with field adaptability. This adjustment is critical in heterogeneous environments, where survival strongly

affects economic returns and operational feasibility and in clonal trials, where individual tree performance may be inflated due to reduced competition in plots with high mortality (Resende *et al.*, 2018; Barbosa *et al.*, 2023). By integrating these two traits, the empirical index provides a more realistic measure of overall performance, reducing the risk of recommending clones that perform well under ideal conditions but fail under stress or site variability.

The practical applicability of this index was validated through on-site field assessments performed during visits to the clonal testing network. However, despite its practical utility, this index should be validated through genetic correlations with key traits or by developing a formal selection index that incorporates economic weights and genetic parameters. Such validation would ensure that the empirical approach aligns with long-term breeding objectives and maximizes genetic gain while maintaining adaptability and sustainability. This is particularly important because trade-offs between productivity and stability are expected in clonal performance: highly productive clones tend to be more sensitive to environmental variation, whereas more stable clones maintain consistent yields across sites. Management practices can also influence SUR variability by modifying stress and competition levels, reinforcing the need to evaluate productivity together with survival under operational field conditions (White *et al.*, 2007).

The selection gains varied depending on the strategy employed. Individual selection per environment yielded higher genetic gains (22.08%, 16.18%, 14.47%, and 12.54% for Usina Cachoeira, Santa Justina, Usina Marituba, and Usina Capricho, respectively) compared to joint selection across all sites (10.26%). These results highlight the importance of site-specific selection in regions with heterogeneous environmental conditions (Reis *et al.*, 2025). The simultaneous selection indices—HMGV, RPGV, and particularly HMRPGV—captured complementary aspects of performance. In the joint analysis, the top-10 lists were largely concordant across indices, and HMRPGV delivered the highest selection gain (41.38%) for MAI × SUR (Figure 4), confirming its suitability when G×E is complex (Ferreira *et al.*, 2023). The prominence of clones CLR 1000 and CLR 1009 across indices underscores their robustness for deployment in Zone 1. By clarifying how genotypes respond across contrasting sites and by prioritizing clones that combine high productivity with survival, our results point toward a deployment strategy that concentrates biomass gains where they are most likely, lessens pressure on native vegetation by supplying a reliable alternative source of wood, and makes more efficient use of available land.

Forest improvement programs in Brazil continuously seek new clonal materials tailored to the diverse regions of the country, focusing on those capable of thriving under the specific edaphoclimatic conditions where they will be planted. Pioneering research that explores new frontiers—particularly in regions with limited representation in the forestry sector—is especially valuable. The presence of genetic materials exhibiting strong stability, adaptability, and productivity underscores the promising potential of the state of Alagoas for the planted forest market, if

areas are properly managed and that the species and hybrids selected are well-suited to the local climate. The selected clones are also undergoing evaluation for resistance to the major diseases affecting eucalypts cultivation, with some already demonstrating notable resistance. These combined efforts are essential for establishing a sustainable and productive eucalypts industry.

As a next breeding phase, we propose complementary actions that can further strengthen the program. These include: (i) expanded clonal tests with a reduced elite set to refine adaptation patterns within each breeding zone, incorporating additional sites that capture within-zone heterogeneity; (ii) pilot operational clonal plantations to validate the MAI × SUR metric under full-scale management conditions, including silvicultural practices, logistical constraints, and exposure to pests and pathogens; and (iii) the evaluation of alternative experimental designs that may enhance both the statistical power and the practical relevance of field trials. In parallel, testing new genetic materials and establishing progeny trials will broaden the genetic base and support long-term improvement. Together, these steps will increase the reliability of recommendations and reduce risks associated with scaling up deployment.

CONCLUSION

The integrated assessment of environments clarified the G×E structure and defined two breeding zones and two mega-environments. This allowed the identification of highly productive and well-adapted clones for Alagoas, with CLR 1009 standing out across sites. Survival-adjusted productivity refined clone ranking, while HMRPGV provided the highest selection gain by integrating performance, stability, and adaptability. These results support more accurate clone deployment and contribute to more sustainable forest planning, promoting efficient resource use and long-term productivity.

ACKNOWLEDGEMENTS

To CNPq for funding the project, to CAPES for the master's scholarship awarded to the first author, and to Clonar Resistência a Doenças. To CNPq and FAPEMIG (APQ-03923-24) for funding the project.

AUTHORSHIP CONTRIBUTION

Project Idea (PI): RFA, ACA, LMSG
 Funding (F): RFA, ACA
 Database (D): RFA, ACA, LMSG, DMM
 Processing (P): TSN, FSA, DAAA
 Analysis (A): TSN, FSA, DAAA, DMM
 Writing (W): TSN, FSA, LMSG, RFA, ACA, DAAA
 Review (R): RFA, ACA, LMSG, DAAA

DATA AVAILABILITY

The datasets supporting the conclusions are included in the article.

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