Received: 12 May 2021 | Revised: 16 Aug 2021 | Accepted: 19 Aug 2021

Electronic Journal of Plant Breeding

Research Article

Selection of clones of *Eucalyptus camaldulensis* (Dehnh.) based on stability for tree volume

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Abstract
A study on the stability of 21 clones of *Eucalyptus camaldulensis* was carried out in four trials established in the southern part of India *viz*., Ariyalur and Marakkanam in Tamil Nadu and Karaikal in Puducherry and Tripathi in Andhra Pradesh during 2010. Trials were laid out in Randomised Block Design (RBD) with four replications each with 16 trees. Single tree volume was estimated after measuring tree height, diameter and form factor after seven years of planting. The volume calculated for individual clones present in all four trials were subjected to AMMI stability analysis using CROPSTAT. The average single tree volume of the clones studied varied from 0.035 to 0.11 m^3 across locations. The Additive Main Effects and Multiplicative Interaction (AMMI) analysis revealed that the genotypic, environmental and GEI effects were 9.6, 68.6 and 21.8 per cent of the total sum of squares, respectively. Planting site plays a major role when compared to genotype and its interaction with the site. IPCA1 captured 75.43 per cent (p=0.001) followed by IPCA2 that captured 19.29 per cent (p=0.007) of the interaction effects. The AMMI Stability Values (ASV) showed a variation from 0.04 to 1.17. The study identified two clones out of twenty-two to be highly stable across the four locations studied for single tree volume.

Key words: *Eucalyptus camaldulensis*, Genotype by Environment Interaction, Single tree volume, AMMI analysis

INTRODUCTION

Tree stem volume is equivalent to the economic value of the trees and hence is widely used by both researchers and forest managers (Arias-Rodil *et al*., 2017; Westfall *et al.,* 2016). Tree volume is the key aspect of growth and yield prediction systems (Valentine and Gregoire, 2001; Özçelik *et al*., 2018; Luoma *et al*., 2019). Forest inventories quantify and assess the volume of the standing crop which in turn is used to estimate yield or productivity (Pereira, 2020; Luoma *et al*., 2019; Setyaji, 2016). Apart from determining the economic potential of the planting material, accurate and up to date information on growth parameters, volume and yield of the available resources help to identify and select superior genotypes for further plantings and clonal deployments. *Eucalyptus camaldulensis* Dehnh. is an important industrial species, used widely for producing pulp in the country (Ginwal, 2014;). There is a wide variation in the yield data available from 12-25 m^3/ha/year to 96 m^3/ha/year and so is the case with corresponding tree volume (Varghese, 2017; Kulkarni, 2013;). Such differences in yield could be attributed to the genetic potential of the planting material, environmental conditions and silvicultural practices. Droughts dramatically reduce eucalyptus productivity (Almeida *et al*., 2010; Scolforo *et al*., 2017; Scolforo *et al*., 2019). Stands of different origin exhibited varied yield patterns (Amateis and Burkhut, 1987). The productivity in eucalyptus can also be enhanced by altering the water and nutrient levels indicating that the potential growth and productivity of clonal eucalyptus plantations varies with the environment and could be manipulated (Stapes *et al*., 2010).

In order to ensure successful plantations, it is imperative to choose the right genotypes with respect to specific environments (Gapare *et al*., 2015). Realising the importance of deploying the best suited clones to a particular site, varied approaches are being used for clonal selections (Almeida *et al*., 2010; Marcatti *et al*., 2017). There is also an increasing consensus on the implications of the genotype interaction effects on clonal selections in tree species (Nunes *et al*., 2002; Goncalves *et al*., 2013; Gapare *et al*., 2015; Scolforo *et al*., 2017). Genotype by Environment (GXE) Interaction in breeding is a result of the varied genetic correlations among the environments (Xie, 2003) and differential responses of specific genotypes to environmental conditions (Paul *et al*., 1997). The presence of GEI indicates that the performance of the clones depends upon the environment and the planting material should be matched to the environment not only to ensure successful plantations but also to minimise the risks in clonal selection (Wahid *et al*., 2012; Xiao *et al*., 2019; Souza *et al*., 2020). Studies on GEI help to identify the environment for a set of genotypes with similar growth and yield performances and also genotypes with lesser interactions that can be deployed to a wide range of environments (Hebert *et al*., 1995; Bentzer *et al*.,1997; Baltunis and Brawner; 2010; Thanh *et al*., 2016). Although manystudies have reported GXE of forest trees, primarily on pines, eucalypts, poplars and spruce (Burdon, 1977; Xie, 2003; Baltunis and Brawner, 2010; Gapare *et al*., 2015; Xiao *et al*., 2019), there are only a few published reports on the stability of clones of *Eucalyptus camaldulensis*. The stability of a genotype refers to its ability to bring about relatively constant yield irrespective of the environment (Sabaghnia, 2006). It may also be perceived as a situation where all the genotypes respond equally to the improved growing conditions (Becker and Leon, 1988). Stable clones ensure guaranteed yield in a set of environmental conditions.

Among the several models used to analyse the stability of genotypes, AMMI (Additive Main Effects and Multiplicative Interactions) is the most commonly used modelfor stability and adaptability of the genotypes and traits across the environment. AMMI is a hybrid model that separates the additive main effects of the genotype and environment by ANOVA and the multiplicative interaction effect of genotypes by environment using PCA (Principal Component Analysis) (Gauch, 1992;). AMMI analyses do not quantify stability or provide any stability measure. Stability alone cannot be used as criteria for selection as the most stable genotypes may not always be good performers. A non-parametrical index that integrates both stability and yield (Farshadfar, 2008; Bose et al., 2014) like AMMI Stability Value (ASV) proposed by Purchase (2000) acts as a stability measure in several GE studies (Bose *et al*., 2014; Adjebeng-Danquah *et al*., 2017; Kumar *et al*., 2018; Sharifi *et al*., 2017; Sabaghnia *et al*., 1995). Genotype Selection Index value (GSI) is an additional parameter to ASV, where the genotypes are re-ranked based on the rank of both yield and stability (Farshadfar, 2008; Sharifi et al., 2017; Załuski et al., 2020; Ajay et al., 2020).

The extend of variation in volume across the test sites and among clones is important for improvement in productivity. The present study seeks to evaluate the effect of GEI on volume and estimate the stability of the *E. camaldulensis* clones for single tree volume using the AMMI model and to identify specific clones suited to each studied test site.

MATERIALS AND METHODS

To assess the clonal stability, trials were established at four different locations; Ariyalur and Marakkanam in Tamil Nadu and Karaikal in Puducherry and Tirupati in Andhra Pradesh (**Table 1**). The clones to be tested were planted during 2010. A total of 21 clones of *Eucalyptus camaldulensis* and one bulk seed lot commonly occurring in the four locations were selected as study material and the necessary observations were taken during 2017. All the trials were laid out in Randomised Block Design (RBD) with four replications each. Each replication had 16 trees, 4 rows at 3 meters spacing with 4 trees in each row at 2 meters spacing. Within each replication, border trees were left out and growth measurements viz., height and girth at breast height (GBH) were measured for 4 central trees in each replication. This was repeated for each of the four replications of all the clones selected for study in each location. A total of 16 trees were observed per clone per location. Tree height was recorded using a clinometer at a distance of 20 m from the base of the tree and the GBH was measured using a measuring tape. The GBH was converted into Diameter at Breast Height (DBH = GBH/π). The diameter of the tree at middle height was

Table 1. Details of the locations of the trials

Locations		Location name	Latitude(N)	Longitude(E)	Altitude (msl)	Rainfall/annum (mm)
Number	Code					
1	A	Ariyalur	11° 09'0. 00"	79°15'00.00"	67	1119.1
2	M	Marakkanam	12° 1'31. 70"	79°56'30.95"	10	1338.0
3	K	Karaikal	10° 55'0. 01"	79°49'59.99"	66	1387.2
4	T	Tirupati	13° 38'7.84"	79°25'11.60"	227	905.0

Table 2. Soil properties of the study environments.

SITE NAME	pH	EC (ds/m)	Bulk density	Organic carbon	Available N (kg/ ha)	Available P (Kg/ ha)	Available K (Kg/ ha)	Soil Texture
Ariyalur	5.6	0.03	1.5	0.8	202.6	21.7	1116.0	Sandy loam
Marakkanam	4.7	0.03	1.7	1.1	156.8	19.1	513.0	Sandy loam
Karaikal	6.1	0.30	1.7	0.6	188.2	19.0	124.0	Sandy clay loam
Tirupati	7.4	0.42	1.1	0.7	373.0	20.2	253.0	Sandy clay loam

measured from the photographs of the trees using a Leica Image analyser (QWin 3.1). Volume was calculated using the $\pi r^2 h$ x form factor. The form factor was estimated for every specific clone taking the billet volume estimated using an image analyser and clone specific form factor was used for volume calculations.

Soil samples collected from all four locations were analysed for the differences in nutrient levels. (**Table 2**). Average rainfall data for 2017 was collected from the Tamil Nadu government data portal (tn.data.gov.in) and the Ministry of Agriculture and Farmers Welfare (agricoop.nic.in).

Statistical analysis of the data for obtaining clonal stability for volume was performed using CROP STAT 7.2. The AMMI model equation given by Gauch and Zobel (1990) is:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

Where Y_{ger} = the observed yield of g^{th} genotype in e^{th} environment for r^{th} replicate; μ = the grand mean; α_g = the deviation of mean of the g^{th} genotype from the grand mean m; β_e = the deviation of mean of the e^{th} environment from the grand mean m; λ_n = the singular value for the n^{th} interaction principal component axis (IPCA); γ_{gn} = the genotype eigenvector for n^{th} (IPCA) axis; δ_{en} = the environment eigenvector values for the n^{th} IPCA axis; ρ_{ge} = the residual effects; and ε_{ger} = the error term.

AMMI STABILITY VALUES (ASV)

ASV is the distance of the coordinate point in a two-dimensional scattergram of IPCA1 scores against IPCA2 scores from zero. The distance from zero is determined by using the theorem of Pythagoras (Purchase *et al.,* 2000). The Genotypic Stability Index (GSI_i) of the i^{th} genotype obtained by the simple addition of the rank value based on the mean volume of the genotype ($RVOL_i$) across environments and the rank of the genotype based on AMMI stability value (ASV_i) given by $GSI_i = RVOL_i + ASV_i$

AMMI Stability Value (ASV)=

$$\sqrt{\left[\frac{SSIPCA1}{SSIPCA2}(IPCA1score)\right]^2 + [IPCA2score]^2}$$

RESULTS AND DISCUSSION

The study aimed to identify the most stable clones across the test environments as well as to find out the optimal clones for specific environments. The average single tree volume of the clones studied varied from 0.035 to 0.11m^3 across locations (**Table 3**). Clones 9 and 196 outperformed all other clones across locations. It is also worthwhile considering the improved seed lots in planting programmes as the bulk seed lot of the orchard was better than many of the clones. The volume recorded in this study for the bulk seed lot was 0.085 m^3 which was above the mean across sites (0.066 m^3) and above all other clones except 196 and 9 Griffin (2014) and Kulkarni (2013) also have made a similar observation on Eucalyptus clones. The results also indicate that Tirupati is the best performing site of all with respect to single tree volume although the rainfall is lesser when compared to other sites. Ariyalur and Marakanam were previously planted with Eucalyptus and Karaikal was previously an agricultural field while Tirupati was virgin soil. The reason for the best performance of the trees in Tirupati could be the virgin soil and fertility level of the deep soil.

The Additive Main Effects and Multiplicative Interaction (AMMI) analysis revealed that the genotypic, environmental and GEI effects were 9.6, 68.6 and 21.8 per cent of the total sum of squares respectively (**Table 4**). Planting sites played a major role when compared to genotype and its interaction with the site. The suitability of the site factors needs to be studied further for yield improvement. The GxE effect was found to be double the genotypic effect which indicated that the present study was appropriate for closer observations on the adaptability of the clones to different study sites. As reported by Gauch (1992 and 2013) high GE sum of squares or GE sum of squares at least equal to that of genotype sum of squares makes the AMMI analysis necessary for multi environmental trials.

AMMI 1 biplot (**Fig. 1**) depicts the principal main effects of genotypes and environment against their respective first multiplicative interactive component (IPCA1). The X axis depicts the genotype and environment main effects for the volume and the y axis depicts the interaction effects. The IPCA1 (Interaction Principal Component Analysis Axis 1) values closer to zero represented high stability and the right side of the x axis represented values higher than the mean. IPCA1 captured 75.43 per cent (p=0.001)

Table 3. Single tree volume (m^3) across the studied sites

Clone	Sites				Clone mean
	Ariyalur	Marakkanam	Karaikal	Tirupati	
7	0.044	-	0.045	0.135	0.056
9	0.079	0.072	0.072	0.184	0.102
10	0.044	0.015	0.016	0.139	0.053
14	0.036	0.014	0.015	0.133	0.049
19	0.087	0.092	-	0.100	0.070
63	0.053	0.037	0.029	0.165	0.071
66	0.057	-	0.031	0.218	0.076
100	0.049	0.026	0.031	0.147	0.063
101	0.073	0.026	-	0.184	0.071
111	0.078	0.050	-	0.111	0.060
115	0.072	-	0.049	0.162	0.071
123	0.050	0.031	0.030	0.131	0.061
124	0.068	0.057	0.065	0.132	0.081
186	0.041	0.037	0.042	0.105	0.056
187	0.032	0.008	0.009	0.090	0.035
188	0.045	0.008	0.009	0.150	0.053
191	0.078	0.050	0.058	-	0.047
196	0.103	0.075	0.081	0.180	0.110
198	0.074	0.050	-	0.112	0.059
301(seed lot)	0.077	-	0.066	0.195	0.085
303	0.043	0.031	0.026	0.159	0.065
307	0.035	0.022	0.016	0.187	0.065
Site mean	0.060	0.032	0.031	0.142	0.066
	SE d	LSD			
Clone	0.003	0.005			
Site	0.006	0.012			
Clone x Site	0.012	0.023			

Table 4. Analysis of variance for the AMMI model

SOURCE	D.F.	S.S.	M.S.	F value	F Prob
Clone	21	0.025	0.001		
Sites	3	0.179	0.060		
Clone X Sites	63	0.057	0.001		
AMMI component 1	23	0.043	0.002	5.39	0.001
AMMI component 2	21	0.011	0.001	3.207	0.007
AMMI component 3	19	0.003	0.000	****	1.000
TOTAL	**87**	**0.261**			

followed by IPCA2 that captured 19.29 per cent (p=0.007) of the interaction effects, indicating that the first IPCA contributes maximum to the interaction sum of squares as observed in several other studies (Anandan *et al*., 2009; Bose *et al*.,2014; Ajay *et al*., 2020). AMMI1 biplot reveals that clones 196 with an average volume of 0.11 m^3 and clone 9 with an average volume of 0.102 m^3 were superior in terms of mean volume as well as stability.

If the genotypes fell almost on the same perpendicular line, they had similar means and those which fell almost on the same horizontal line had similar interaction patterns. Accordingly, clones 19,303,115 and 101 were found to have similar means and clones 196, 14,7,10,100,9, 10 and 303 similar interaction patterns. The ideal genotypes for cultivation are those with higher mean coupled IPCA1 values near unity (Koundinya, 2019). In the present study, clone 196 and clone 9 can be considered stable across sites and recommended for mass plantation. Tirupati was observed to be a favourable site while Marakanam and Karaikal were observed to be poor and sites Ariyalur as an average site. The biplot revealed that clones 198, 111 and 123 showed specific adaptability to Ariyalur; 191 to Marakanam; and 187 to Karaikal.

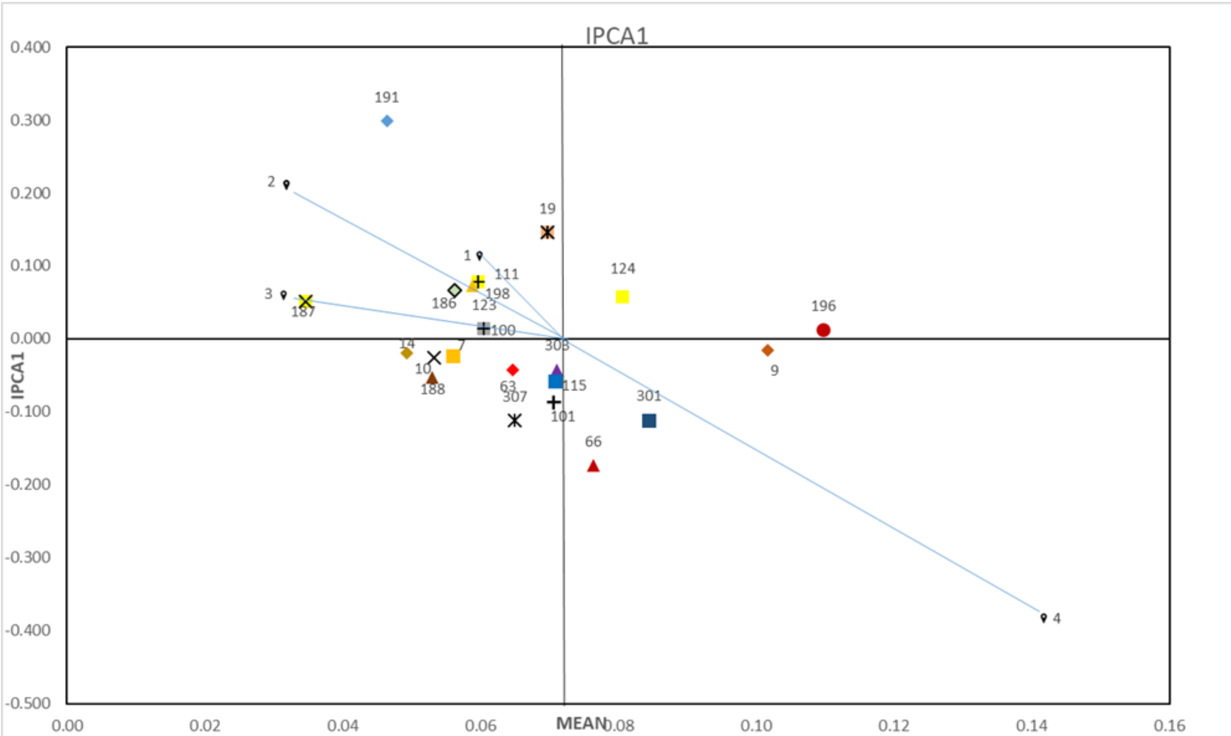

Fig.1. Ammi Biplot 1; IPCA1 Vs MEAN

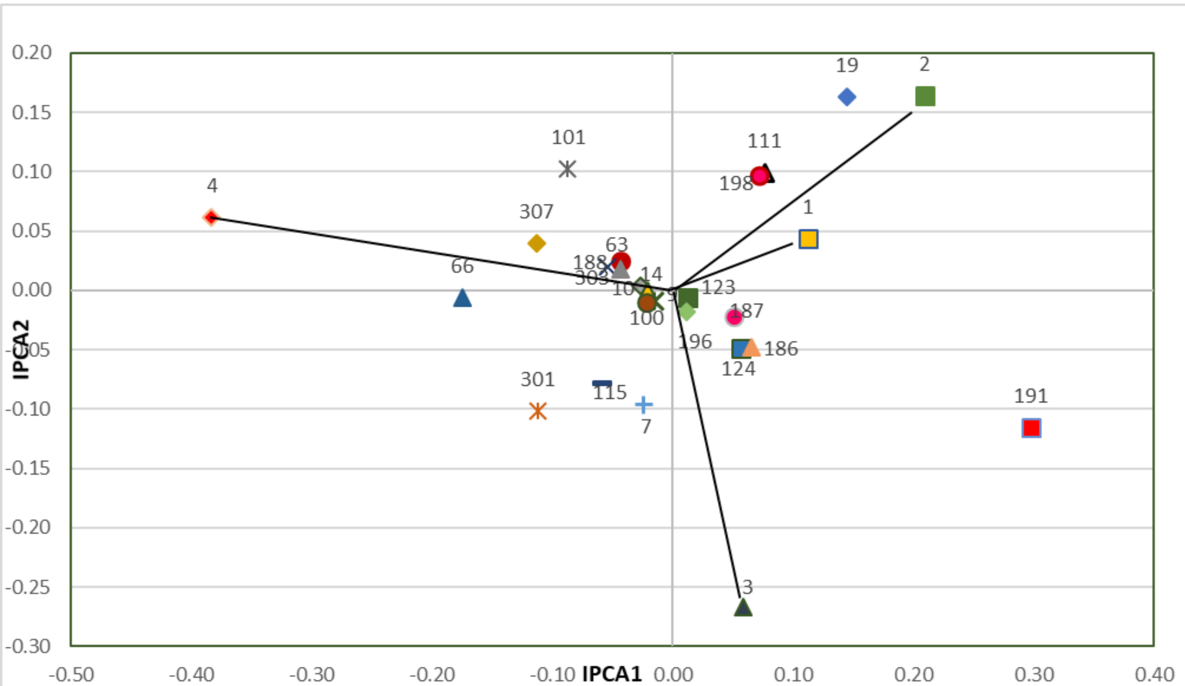

Fig. 2. AMMI BIPLOT 2; IPCA1 Vs IPCA2

Farthest the distance between the marker and its origin in the biplot 2 (**Fig.2**) as it is observed for Tirupati and Marakanam in the present study showed that the two are the most discriminating environments. Vector angles more than 90^0 between any two environments in the biplot 2 indicated that the clonal responses between these environments were negatively correlated (Olivoto *et al.*, 2019) or unstable in their performance (Tena *et al.*, 2019). The performance of clones 307 and 101 was negatively correlated with clones 198 and 111 between Marakanam and Tirupati where vector angles exceeded 90 degrees (**Fig.2**). The performance of clones 198 and 111 was superior at Marakanam; at the same time 307 and 101 were superior in Tirupati and comparatively their performance in vice versa sites was poor.

Clones 191, 19 and 66 were far away from the centre of the biplot and hence are expected to be site specific clones. Based on their proximity to the environments, clone 191 is adapted to Karaikal, Clone 19 adapted to Marakanam and Clone 66 is adapted to Tirupati. Lesser angles and clustering of the clones in a quadrant corresponding to the environment suggests the similarity in performance of the clones in that particular environment. IPCA2 which projects the environmental vectors and genotypes in the four quadrats of the biplot helped to identify the most adaptable genotype to a specific environment (Sharifi *et al.*, 2017).

Observations shown in the biplot were confirmed by the ASV (AMMI Stability Values as shown in **Table 5.** ASV showed a variation from 0.04 to 1.17. Clone 191 showed poor volume and stability while clone 196 ranked high for both mean volume as well as its stability. Low ASV values and GSI indicated high clonal stability across environments (Kang 1988; Kang 1991; Farshadfar, 2008; Zaluski *et al.*, 2020). On the contrary, genotypes with low stability values along with low mean are considered undesirable (Gauch and Zobel, 1990; Bose *et al.*, 2014). In the present study, clones 196 and 9 are the most desirable clones with high mean volume and high stability. On the other hand, a seedling origin seed source (301) in spite of the high mean volume, did not exhibit stability as per GSI.

It is concluded that the studied environments influenced the single tree volume and in turn the yield of *E. camaldulensis* plantations. The results also indicated that Tirupati was the best environment with respect to single tree volume for the clones studied. AMMI stability values and GSI could be used for discerning clones 196 and 9 as the most stable clones. Tirupati and Ariyalur were the two environments to which the clones responded differently whereas, the clonal performances were highly similar in the other two environments. The species is heavily depended upon for paper production by industries in the country. Where there is not adequate production and an avid requirement to improve the quality and quantity of the raw materials, selection of planting material is essential. The presence of GEI in clonal trials results in ambiguous

Table 5. Mean Volume, AMMI stability Values (ASV) and Genotype Selection Index (GSI) for the Clones

Variety\ Site	Clone Mean Volume (m^3)	Mean Vol (Ranking)	IPCA1	IPCA2	ASV	Ranking (ASV)	GSI
7	0.056	17	-0.025	-0.096	0.136	7	24
9	0.102	2	-0.016	-0.008	0.063	3	5
10	0.053	18	-0.026	0.004	0.103	6	24
14	0.049	20	-0.020	0.000	0.080	4	24
19	0.070	9	0.146	0.163	0.591	20	29
63	0.071	6	-0.043	0.024	0.170	9	15
66	0.076	5	-0.174	-0.007	0.681	21	27
100	0.063	12	-0.021	-0.010	0.084	5	17
101	0.071	8	-0.087	0.102	0.354	17	25
111	0.060	14	0.077	0.099	0.317	16	30
115	0.071	7	-0.058	-0.078	0.241	13	20
123	0.061	13	0.013	-0.007	0.052	2	15
124	0.081	4	0.057	-0.049	0.230	12	16
186	0.056	16	0.066	-0.048	0.263	14	30
187	0.035	22	0.051	-0.023	0.200	10	32
188	0.053	19	-0.054	0.020	0.210	11	30
191	0.047	21	0.298	-0.116	1.171	22	43
196	0.110	1	0.012	-0.019	0.050	1	2
198	0.059	15	0.072	0.097	0.298	15	30
301	0.085	3	-0.112	-0.102	0.449	19	22
303	0.065	11	-0.043	0.017	0.170	8	19
307	0.065	10	-0.113	0.039	0.442	18	28

phenotypic expressions and misinterpretations about the genetic effect thus making selections challenging. The present study showed that an understanding of GEI in *E. camaldulensis* is essential for high and reliable yield before the clones are deployed. Furthermore, where a sufficient number of tested genotypes are available and where the area to be planted is characterised by varied agroclimatic conditions, the grouping of clones and matching them to specific areas would prove to be a more efficient breeding strategy. It would also provide better yield and improved economic returns. More environments and more genotypes could also be explored for identifying site specific clones.

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