PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(2): 1-7
https://doi.org/10.14719/pst.6227

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RESEARCH ARTICLE

Assessing the superiority of *Casuarina equisetifolia* L. and *Casuarina junghuhniana* clones for dendroenergy under Semi-Arid condition

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OPEN ACCESS

ARTICLE HISTORY
Received: 21 November 2024
Accepted: 23 January 2025
Available online
Version 1.0 : 04 March 2025
Version 2.0 : 01 April 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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CITE THIS ARTICLE
Nayak A, Ramesh KR, Parthiban KT, Devanad PS, Kumar P, Selvan T, Sivaprakash M, Sivakumar B, Bhuvaneswari SSB, Patil P, Riyaz A, Rajah RA. Assessing the superiority of *Casuarina equisetifolia* L. and *Casuarina junghuhniana* clones for dendroenergy under Semi-Arid condition. Plant Science Today. 2025; 12(2): 1-7. https://doi.org/10.14719/pst.6227

Abstract

This study assessed genetic variation and heritability in 13 clones of casuarina species for growth attributes. The experiment, conducted in Coimbatore, India, used a Randomized Block Design (RBD) with three replications. Biometric parameters, including plant height, collar diameter, sturdiness quotient and volume index, were measured 2, 4 and 6 months after planting (MAP). We observed significant variations among the clones for all traits. Clone E9B consistently outperformed others across growth stages, particularly in plant height, collar diameter and volume index. Conversely, clones CE04 and CE 06 generally underperformed. Genetic variability analysis revealed a high PCV and GCV for the volume index, indicating good potential for selection and improvement. Plant height exhibited exceptionally high heritability (99.4 %), suggesting minimal environmental influence. Association studies showed strong positive correlations between plant height, basal diameter and volume index at phenotypic and genotypic levels. Path analysis identified basal diameter as having the highest direct positive effect on volume index. Diversity analysis clustered the clones into three groups, with plant height contributing 58.97 % to genetic divergence. These findings highlight substantial genetic variability among the studied casuarina clones for key growth traits. The superior performance of E9B across multiple attributes suggests its potential value for breeding programs or direct use in plantations. High heritability of plant height and strong correlation between growth traits indicate promising prospects for genetic improvement through selection. This research provides valuable insights for casuarina breeding and selection programs to develop improved varieties for agroforestry and plantation forestry applications.

Keywords

associations studies; casuarina; diversity analysis; GCV; heritability; PCV

Introduction

The family Casuarinaceae, which includes multifunctional tree species like casuarinas, are extensively distributed in Southeast Asia, Australia, and the Pacific islands. In the world's tropical and subtropical climates, plants of the Casuarina family, one of the four genera of Casuarinaceae, are widely grown. The most commonly imported, grown, and subjected to tree development programs are *C. equisetifolia, C. junghuhniana, C. cunninghamiana, C. cristata, C. obesa,* and *C. glauca*. A species is chosen based on its capacity to adapt to various ecological conditions, resistance to salty alkali and drought, and early, fast development (1). Casuarinas are frequently planted in the tropics, subtropics, and Mediterranean regions. Of the 96 species of trees in the Casuarinaceae family, *Casuarina equisetifolia* has drawn the most attention because of its various applications. Still, more recently, *C. junghuhniana* has also acquired popularity due to its flexibility and utility (2).

Genotype x environment interaction, where genetic performance varies across different environments, is a significant challenge in tree improvement. To mitigate this, genetic studies are often conducted in diverse settings. (3). Improved genotypes with consistent performance across environments are a primary goal in tree improvement (4). Although stability analyses of crops have been carried out extensively, reports of these studies on tree species are few (5).

Rapid economic expansion, industrialization, and population growth drive the nation's demand for forest goods and services. Wood has emerged as one of the most significant forest products in recent years and is a crucial raw resource for companies that rely on forests. The poor productivity of Indian forests means that wood-based paper mills in India still have trouble obtaining raw materials from the forests. The industry uses agricultural forestry and social forestry plantings to supply wood and fulfil the rising demand for timber in a sustainable manner (6). Significant variations for the variables under research at each location were found among the clones based on the variance analysis. There were notable variations in the clones' development and quality attributes within and across locations.

India leads the world in casuarina production, with an estimated 800000 hectares of plantations. Casuarina is valued for its potential as an agroforestry tree species. An estimated 500,000 hectares of casuarina plants are spread over the Union Territory of Puducherry, the states of Andhra Pradesh, Orissa, and Tamil Nadu. In India, *Casuarina equisetifolia* L. is the species most commonly planted with casuarina, because of its quick rotation and ongoing demand in the building sector, such as scaffolding and low-cost building materials (6).

According to some research, all tree parts, including the leaves, bark, seeds, flowers (aerial parts), and roots, can be used to treat various illnesses and disorders. They also exhibit a variety of pharmacological and medicinal properties, including hepatoprotective, anti-inflammatory, anti-cancer, anti-bacterial, anti-fungal, and anti-oxidant activities, as well as hepatoprotective, anti-diabetic, anti-hyperlipidaemic, and anti-ulcer actions. Additionally, the tree bark can treat headaches, toothaches and stomachaches (7).

The dendroenergy sector, which harnesses energy from woody biomass, holds significant potential for sustainable energy production. However, research gaps like advanced conversion technologies, sustainable plantation management and harvesting, biochemical conversion, and integration with existing energy systems hinder its full realization. In this context, the present study has been designed to identify Casuarina clones with higher biomass yields and enhanced thermo-chemical properties for clean and sustainable energy conversion.

Materials and Methods

The raw materials for this investigation included 13 clones of Casuarina species selected from Forest College and Research, Mettupalayam, Tamil Nadu. The biometric data, *viz.,* height collar diameter, were recorded and were subjected to genetic diversity analysis, association studies, path analysis and cluster analysis.

Experimental area

The selected clones were raised at Syndicate Private Industry, Jadeyampalayam, Annur, Coimbatore, Tamil Nadu, India, to develop a high-density energy plantation model by deploying thirteen clones of both Casuarina species *viz Casuarina junghuhniana, Casuarina equisetifolia* with *casuarina junghuhniana* (MTP 3) as a check variety (Table 1).

Germplasm material

The rooted cuttings of thirteen casuarina clones (10 - *C. junghuhniana* and 3 - *C. equisetifolia*) developed by the Forest College and Research Institute, Mettupalayam, were examined in the present investigation and their details are furnished in Table 1. The rooted cuttings were grown in polybags for 3 months in the Forest College and Research Institutes' hi-tech nursery to acclimatize them to local climatic conditions. The 3-month-old plants about 40-70 cm tall were selected for field planting.

Experimental design and layout

The planting was done in January 2024 with a Randomized Block Design (RBD). The trees were planted at a spacing of 1.5 x 1.5 m^2 distance between each row. The experiment was conducted in three replications, each with nine seedlings from each clone. Regular watering schedules and weeding treatments were done as per the need.

Table 1. List of selected thirteen clones of casuarina

Sr. No.	Species	Clone name
1	*Casuarina junghuhniana*	A 01
2	*Casuarina junghuhniana*	A 06
3	*Casuarina equisetifolia*	CE 04
4	*Casuarina equisetifolia*	CE 06
5	*Casuarina equisetifolia*	CE 08
6	*Casuarina junghuhniana*	CJ 02
7	*Casuarina junghuhniana*	CJ 03
8	*Casuarina junghuhniana*	E 2
9	*Casuarina junghuhniana*	E 5
10	*Casuarina junghuhniana*	E 9B
11	*Casuarina junghuhniana*	E 10A
12	*Casuarina junghuhniana*	E 13
13	*Casuarina junghuhniana*	MTP 3 (Check)

Observations recorded

Observations on quantitative traits *viz.,* plant height (cm), collar diameter (cm), sturdiness quotient, and volume Index were recorded using a measuring scale and Vernier calliper. The data were recorded at different growth periods.

Quantitative traits

The observations, like plant height and collar diameter, were recorded at different periods, 2 months after planting (MAP), 4 MAP and 6 MAP. The Sturdiness quotient was calculated using the Equation 1(8) formula.

$$\text{Sturdiness quotient} = \frac{\text{Height (cm)}}{\text{Diameter (cm)}}$$

The volume index was arrived at by using the Equation 2 formula (8).

$$\text{V1} = \frac{(\text{Collar diameter})^2}{\text{Height}}$$

Data analysis

The raw data was inputted into Excel and then transferred to the GENERS statistical software for genetic variability, association studies, path analysis and genetic diversity analysis. GENERS is a software tool for genetic research (9). It analyses and processes data using various statistical models to improve plant breeding.

Results and Discussion

Significant variations in the variables under investigation were found between the clones according to the variance analysis. There were notable variations in the clone development concerning height and collar diameter at different periods. Casuarina is a promising tree species for agroforestry due to its potential for rapid growth and significant height and diameter at breast height (DBH).

Plant Height

The investigation revealed a significant variation among the selected clones of casuarina species at different growth heights was recorded as maximum by the E9B clone at all the three different growth periods *viz* 36.7 cm at initial, 94.6 cm at 2 MAP, 180.6 cm at 4 MAP, 230.4 cm at 6 MAP followed by clone E2 with 31.06 cm, CJ02 with 30.2cm and E5 with 30.16 cm. Whereas clone CE 04 recorded the lowest plant height when compared to the general mean *viz* 21.01 cm, followed by clone CE06 (21.12 cm), E10 A (21.35 cm) (Fig. 1).

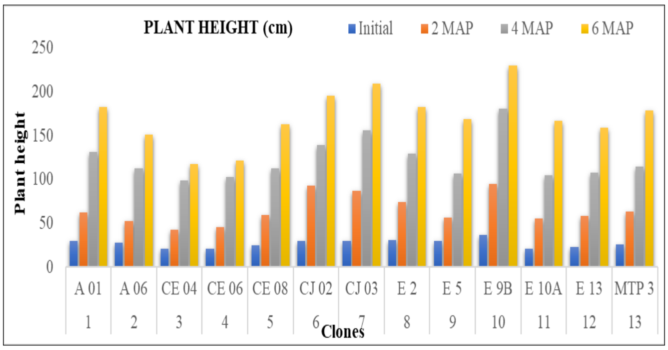

Fig. 1. *Casuarina* clonal variation for height at different growth periods.

At 2 MAP, 4 MAP and 6 MAP, E9B recorded the highest plant height (94.6 cm, 180.6 cm and 230.4 cm), whereas CE04 recorded the lowest plant height (42.5 cm at 2 MAP, 99.3 cm at 4 MAP) and CE06 (121.9 cm at 6 MAP).

Research indicates that eight casuarina hybrid clones, notable height was observed in CH2, CH4 and CH5 clones (2.68, 2.72 and 2.67 m, respectively) at 9 MAP (10). Significant differences were noted between *Casuarina junghuhniana* clones regarding survival, height, diameter and stem volume at six years of age. They suggested eleven clones intended for industrial farming(11). Research indicates the assessment of twelve clones of six-year-old Casuarina trees in the provinces of Guangdong and Hainan. The trees' diameters ranged from 11.4 to 14.3 cm, while their heights ranged from 13.9 to 16.3 m (12). Clonal variation in Casuarina exposed to sodium chloride in nursery circumstances was also noticed (13). Previous research on *Populus deltoides*, Eucalyptus clones, *Eucalyptus tereticornis* and Casuarina has found growth variations among different genotypes (14-17).

Collar Diameter

There were notable differences in collar diameter across different clones at all growth stages studied. The clone E9B recorded the highest collar diameter across all the growth periods viz., 0.4 cm - initial, 0.85 cm - 2 MAP, 1.9 cm - 4 MAP and 2.02 cm - 6 MAP. The clone CE 04 registered the least collar diameter at all the growth periods, viz., 0.22 cm at initial, 0.52 cm at 2 MAP, 0.91 cm at 4 MAP and 1.05 cm at 6 MAP. Hence, E9B recorded the highest collar diameter and CE 04 recorded the lowest collar diameter compared to the general mean (Fig. 2).

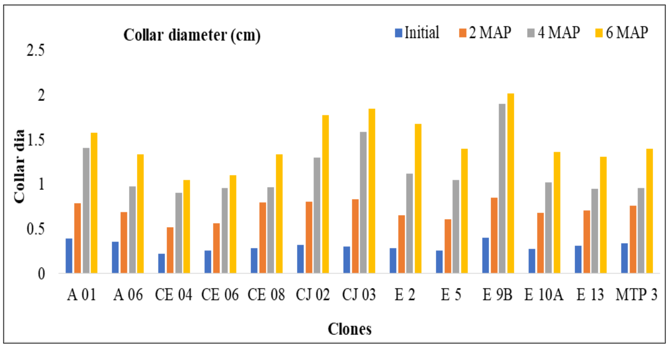

Fig. 2. *Casuarina* clonal variation for basal diameter (cm) at different growth periods.

The research examines the impact of fertility on nutrients in *Mitragyna speciosa* and discovered a noteworthy growth in plant diameter with increased fertilizer application. The diameter development of MD KP01 in 12MAP was the highest, followed by MP 01 and LL 15. 4.84, 4.58, and 4.49 cm, respectively (18). Similar findings for the diameter of nine-month-old Melia clones and productivity in height and volume. The least-diameter growth was 2.32 cm, as observed in CJ01 (19). The results of a research study revealed that the dbh growth of clones of *Casuarina junghuhniana* was barely 6.5 cm (20).

Sturdiness Quotient: The study found that the sturdiness quotient varied significantly among different clones at different growth stages. At the initial stage, CE 06 recorded the highest sturdiness quotient, followed by clones E5

(100.53), E2 (100.19) and CE 04 (100.05). Whereas E 13 recorded the lowest sturdiness at the initial stage of planting (73.58) (Fig. 3). At 2 MAP, CJ 02 recorded a high (115.06) and CE 08 registered a low (74.84) sturdiness quotient (Fig. 3).

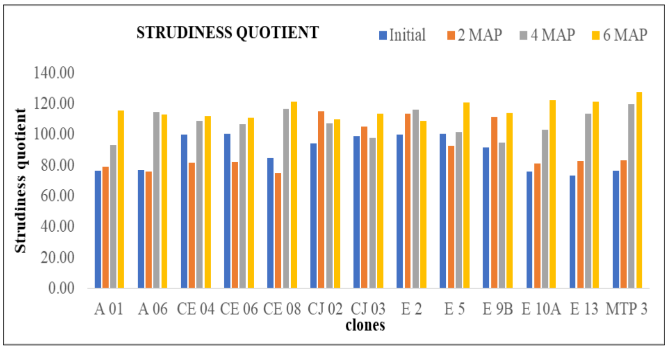

Fig. 3. *Casuarina* clonal variation for sturdiness quotient at different growth periods.

At 4 MAP, MTP 3 (Check variety) recorded a maximum of 119.79 and A 01 recorded a minimum of 93.05. During the last growth period (6 MAP), the highest was recorded by MTP 3 (127.79) and the lowest was recorded with E2 (109.05). Sturdiness and growth of seedlings in a nursery have a positive bearing on the establishment of growth of seedlings in plantations (21). Higher densities result in taller seedlings, whereas lower densities, as seen in *Pinus taeda* L., affect root collar diameter, dry weight, and sturdiness quotient values (22). Research indicates that the value of the sturdiness quotient did not depend on the seedling's height. Still, it varied during the production season in *Quercus robur* L. during the growing season (23). Different growing media's effect on teak growth performance was investigated (24). The results revealed that at the end of 150 days of growth, the sturdiness quotient in every growing medium-aside from sawdust: soil: FYM (1:1:1) crossed the limit (6.0), indicating that continued seedling growth in the container would put survival and field growth at risk.

Volume Index

There were significant differences ($P < 0.05$) in the Volume Index among the 13 clones examined at various growth stages. The clone E9B registered higher volume index during all the growth periods *viz.,* (5.87) initial stage, (68.35) - 2 MAP, (651.97) - 4 MAP and (940.12) 6 MAP whereas clone CE 04 recorded lowest volume index during all growth periods viz., (1.02) initially, (11.49) 2 MAP, (82.23) 4 MAP and (129.87) 6 MAP (Fig. 4).

Similar studies indicate the performance of 14 hybrid poplar clones, whereas clones R-247, DN-70 and Simplot

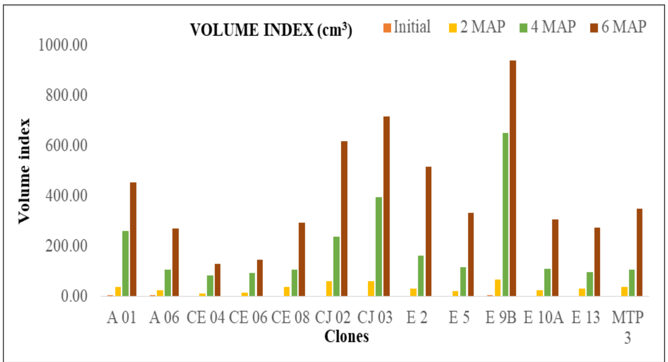

Fig. 4. *Casuarina* clonal variation for volume index at different growth periods.

had the highest tree volume index (25). The study concluded that the clones with the most extensive mean tree volume index are well-suited to the environment and show the most significant promise for high biomass yield. However, the clones with the lowest tree volume index will not be suitable for biomass production in the particular region.

Variability studies

The variations among the clones in terms of their performance were estimated for phenotypic and genotypic variances using four traits, viz., height, collar diameter, sturdiness quotient and volume index. The phenotypic coefficient of variation was higher compared to the genotypic coefficient variation for all four traits. The phenotypic variation coefficient was higher than the genotypic variation coefficient for all four traits. This suggested that non-additive gene action affected these characteristics (26). The phenotypic coefficient of variation and genotypic coefficient variation for plant height varied from 20.6 %and 20%, respectively. Low genetic variation for height has been observed in *Bambusa pallida* and *E. tereticornis* (27, 28). Whereas, in the case of heritability, the plant height recorded higher heritability with 99.41 % when compared with the general mean of 41.08 %(Table. 2).

The collar diameter recorded a low phenotypic variation of 14.55 %and a low genotypic variation of 12.40%. Heritability was 72.67 %when compared to the general mean of 21.78%. The sturdiness quotient registered a low PCV of 11.19 %and GCV of 8.99%, along with higher heritability of 64.62 %compared to the general mean of 14.89%. In the volume index, a high PCV was recorded at 48.57 %and a high GCV of 44.73%. Heritability was recorded higher for this trait with 84.84 % (Table. 2). A similar study on *Casuarina equisetifolia* found that volume index and frustum volume showed the highest genetic variation among 23 provenances at the age of 3 years (29). Tree height exhibited the least genetic variability, with low PCV and GCV values (5.93 % and 27.2 %). The frustum of volume displayed the

Table 2. Genetic estimates for growth attributes

Traits	PCV (%)	GCV (%)	Heritability (%)	GA (%) of mean
Plant height	20.5958	20.0004	99.4082	41.0787
Collar diameter	14.5452	12.3996	72.6738	21.7753
Sturdiness Quotient	11.1873	8.9931	64.6204	14.8923
Volume Index	48.5680	44.7342	84.8355	84.8781

highest heritability (0.24), followed by diameter at breast height, basal diameter, and volume index (0.20).

Analyzing the components of phenotypic variance is more helpful in understanding the factors influencing variation. The heritable genetic variance can be utilized for future breeding programs. Heritability measures how well genotype selection is based on phenotype but does not represent genetic advancement (30). Therefore, higher heritability estimates may not always translate into higher gains (31). The best outcomes of the genetic advancement that can be anticipated from selection would come from combining the genotypic variation coefficient with heritability estimations (32).

Association studies

The phenotypic and genotypic correlations of quantitative traits with volume index showed significant positive and negative variation, as furnished in (Table 3). The height exhibited positive correlation with basal diameter (phenotypic - 0.9139 and genotypic - 0.9698), with sturdiness quotient (0.3316 - phenotypic and 0.5069 - genotypic) and volume index (0.9164 - phenotypic and 0.9544 - genotypic).

Table 3. Phenotypic and genotypic correlation coefficient of growth attributes

Characters		Plant height	Basal diameter	Sturdiness quotient	Volume index
Plant height	P	**1.000**	0.9139	0.3316	0.9164
	G	**1.000**	0.9698	0.5069	0.9544
Basal diameter	P		**1.000**	-0.0688	0.9792
	G		**1.000**	0.2769	0.9946
Sturdiness Quotient	P			**1.000**	0.0074
	G			**1.000**	0.2350
Volume Index	P				**1.000**
	G				**1.000**

The Basal diameter registered a negative phenotypic correlation with the sturdiness quotient (-0.0688), whereas it recorded a positive genotypic correlation (0.2769). With the volume index, the positive phenotypic and genotypic correlation was recorded (0.9792 and 0.9946, respectively). The sturdiness quotient recorded both phenotypic and genotypic positive correlation with volume index (0.0074 and 0.2350).

Tree height and diameter at breast height were positively correlated in *E. camaldulensis* (33). A similar study on the *Santalum album* also found a highly significant and positive correlation between volume and basal diameter (34). These findings indicate a strong inherent relationship between different traits, supporting the results of this study and previous research (35).

Path coefficient analysis

The assessment of the direct and indirect impacts of morphometric trait attributes on volume is given in Table 4. Plant height exhibited a direct negative effect of (-0.0970). Meanwhile, the sturdiness quotient registered a positive effect of basal diameter at 1.0939 and a negative impact of -0.0188 (Table 4). The residual effect was recorded as 0.0947.

***Indirect effect*:** The plant height had an indirect positive impact on volume index through basal diameter (1.0609) and a negative effect through sturdiness quotient (-0.0095). At the same time, basal diameter indirectly negatively impacted volume index through plant height (-0.0941) and sturdiness quotient (-0.0052). In the case of sturdiness quotient, it had an indirect negative effect on volume index through plant height (-0.0492) and had a positive indirect effect through basal diameter (0.3029) (Table. 4).

Table 4. Path coefficient analysis of growth attributes on Volume Index

Traits	Plant height	Basal diameter	Sturdiness quotient
Plant Height	**-0.0970**	1.0609	-0.0095
Basal diameter	-0.0941	**1.0939**	-0.0052
Sturdiness Quotient	-0.0492	0.3029	**-0.0188**

In contrast to agriculture, publications on path analysis in forestry are only accessible for a restricted number of species. Studies have been done on the following: *Pseudotsuga menziessii*, *Tsuga heterophylla*, *Picea glauca* and *Alnus crispa*, *Populus species*, *Santalum album*, *Dalbergia sissoo*, *Terminalia tomentosa*, *T. arjuna*, *Spondias tuberosa*, *Casuarina equisetifolia* and *Pinus gerardiana* (34-44).

Diversity analysis

The genetic diversity analysis revealed that thirteen clones were resolved into three clusters with cluster I being the largest with seven clones (A01, CJ02, CJ 03, E2, A06, E5, MTP 3), followed by cluster II with five clones (CE 08, E 10A, CE 06, CE 04, E13) and the least was recorded by cluster III with only one clone (E9B) (Table 5).

The cluster mean values for the traits were estimated and furnished in (Table 6). The highest cluster mean was observed in Cluster III for plant height (79.25), followed by Cluster I (65.48) and the lowest cluster mean value for plant height was observed in Cluster II (48.32). For basal diameter, the highest cluster mean was observed in cluster III (0.98), followed by cluster I (0.75) and the lowest cluster mean for basal diameter was noticed in cluster II (0.62).

In the case of sturdiness quotient, the highest cluster mean was recorded by cluster I (88.53) followed by cluster III (81.47) and the lowest cluster mean was observed in cluster II (81.4). The volume index also showed variations concerning cluster means. The highest cluster mean of volume index was recorded in cluster III (76.59), followed

Table 5. Clustering pattern of Casuarina clones for growth attributes

Cluster No.	Number of clones	Members
I	7	A 01, CJ 02, CJ 03, E 2, A 06, E 5, MTP 3 (Check)
II	5	CE 08, E 10A, CE 06, CE 04, E 13
III	1	E 9B

Table 6. Cluster mean values for growth attributes

Cluster	Plant height (cm)	Basal diameter (cm)	Sturdiness quotient	Volume index (cm^3)
I	65.4814	0.7491	88.5329	37.6067
II	48.3173	0.6013	81.3700	17.9553
III	79.2533	0.9800	81.4667	76.5900

Table 7. Percent contribution of growth attributes to genetic divergence

Characters	No. of first rank	% Contribution
Plant height	46	58.97434
Basal Diameter	8	10.25640
Sturdiness Quotient	2	2.56410
Volume index	22	28.20512
Total	**78**	**100**

by cluster I (37.61) and the least was recorded by cluster II (17.96). The highest percentage of divergence was given by plant height (58.97 %), followed by volume index (28.20 %), basal diameter (10.25 %) and sturdiness quotient (2.56 %) (Table 7).

Similar studies were conducted where nine clones of Casuarina were evaluated in arid and semi-arid conditions (45). Under arid conditions, the clones were grouped into two clusters; Cluster 1 (Cluster 1A – I, H and F genotypes; Cluster 1B- C and D genotypes) and Cluster 2 (A, B and E). Under semi-arid conditions, Cluster 1 comprised of five clones viz., E, D, A, B and C whereas Cluster 2 contained clones F and H. Divergence analysis is crucial for determining the genotypes needed for hybridization. Three key factors must be considered when choosing genotypes: which specific cluster to use as parents, which genotype to choose from the selected clusters, and how much each character contributes to overall divergence (44).

Many researchers noted that there were notable variations in different tree species, such as Acacia nilotica and that some provenances, seed sources, and progenies were superior (45). Similar studies on *Eucalyptus camaldulensis*, *Tecomella latifolia*, *Terminalia arjuna*, *Lagerstroemia speciose*, *Dalbergia sissoo*, *Pinus elliottii* var. *elliottii* (50), *Leucaena leucocephala*; *Jatropha curcas*, *Pinus kesiya* and *Populus alba* Linn. further support the present conclusions (33, 46-54).

Conclusion

Clone E9B consistently outperformed others across all growth stages for most traits, particularly excelling in plant height, collar diameter and volume index. The superior performance of E9B across multiple attributes suggests its potential value for breeding programs or direct use in plantations. High heritability of plant height and strong correlations between growth traits indicate promising prospects for genetic improvement through selection. Future research in casuarina breeding could include long-term field trials, molecular characterization using DNA markers, and crossbreeding with the superior E9B clone. Advanced breeding techniques like genomic selection could accelerate genetic improvement.

Acknowledgements

All the authors are very grateful to the Forest College and Research Institute, Mettupalayam and Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, for their support and guidance. We want to thank the Biotherm Industry for providing their valuable insights.

Authors' contributions

AN conducted the research, analyzed the data and wrote the manuscript. KRR, KTP, PSD and PK contributed to the initial concept, oversaw the research, and investigated relevant areas. KTP helped design the study. TS, MS, BS and SSBB reviewed and edited the manuscript. PP, AR and RAR assisted with data analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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