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## Research Article

### Genetic variability in international provenances of *Casuarina equisetifolia* L.

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#### Abstract

*Casuarina equisetifolia*, the most commonly grown species of *Casuarina* in India, is mainly used for pulpwood, scaffolding, poles, fuelwood and stakes for banana crop. It is most suited for agrarian ecosystems due to many favourable characteristics like desirable stem form and fast growth. It is one among the non-leguminous nitrogen fixing tree species also. In India, tremendous variations are documented for tree species in different states. Wide variations were observed when 23 international provenances of *C. equisetifolia* infused from CSIRO, Australia were subjected to multi-location field testing. Among the three primary characters which contribute directly towards volume growth, diameter at breast height showed higher degree of variation than total height in all the provenances. Volume index recorded the maximum genotypic coefficient of variation followed by frustum volume in all the provenances at 3 years of age. Tree height registered the lowest value for GCV and PCV. Among the various characters studied, frustum volume recorded the maximum heritability (0.24) followed by diameter at breast height, basal diameter and volume index (0.20). The lowest value was for tree height (0.05). Mahalanobis'  $D^2$  statistics followed by Tocher's clustering method grouped the 23 provenances of *C. equisetifolia* into 9 clusters with cluster strength varying from one (clusters 8 and 9) to eight (cluster 4). Clusters 4,5,7 and 9 registered higher mean values for frustum volume and volume index. Based on the genetic variability studies, it is concluded that provenances 18141 Kenya, 18378 QLD, 18298 Thailand, 18344 Malaysia, Mixed Seedlot India, 18586 China and 18355 Benin could be the potential candidates for future breeding programmes.

**Keywords:** *Casuarina equisetifolia*, Variability Studies, Genetic Divergence, Tree Breeding

#### INTRODUCTION

In India, the most widely grown species of *Casuarina* is *C. equisetifolia* L. Multiple utility values of this farmer friendly tree species make it suitable for agrarian ecosystems. It is mainly used for pulpwood, scaffolding, poles, fuelwood and stakes for banana crop. In barren areas where majority of the plant species do not grow, *Casuarina* spp. thrive and turn them productive (Subbarao and Rodriguez-Barrueco, 1995). It is also being utilized for rehabilitation of salt affected lands. Extensive shelterbelt plantations of this species are raised in the coastal belts of all the southern states and West Bengal and Odisha in particular as few rows of this tree species can reduce the wind velocity to a great extent. Its nitrogen fixing ability, desirable stem form, fast growth and light crown

characteristics make it an ideal tree for agroforestry systems (Viswanath *et al.*, 2001). With an estimated 800,000 ha of plantations, India is the largest *Casuarina* growing country in the world (Pinyopusarek and Williams, 2000).

In India, tremendous variations are documented for tree species in different states. These variations are mainly for characters like branch angle, length of branchlets, shape of crown, size and shape of cones and seeds (Kondas, 1983). Many researchers have reported abundant variations in its growth parameters also (Kumar and Gurumurthi, 1998; Prasad and Dieters, 1998).

As *C. equisetifolia* is an exotic species to India, it is essential to infuse new germplasm from its native ranges to broaden the genetic base. Therefore, ICFRE-Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, India imported 39 international provenances of this species and multi-location provenance trials were established with select 23 provenances. The present study is aimed to understand the variation in these germplasm and to identify genetically diverse provenances for future breeding programmes.

## MATERIALS AND METHODS

The experimental materials comprised of 23 international provenances of *Casuarina equisetifolia* imported from CSIRO Australia. These provenances were established in a Randomized Complete Block Design with five replications (10 tree plot) at a spacing of 2m x 2m at the Neyveli Field Research Station, Tamil Nadu (Latitude: 11°32'20.33" N and Longitude: 79°28'45.79" E, Altitude 35 m), of the ICFRE - Institute of Forest Genetics and Tree Breeding, Coimbatore. Observations recorded on tree height, diameter at breast height (DBH), basal diameter (BDM), frustum volume (FV) and volume index (VI) at 3 years of age were used for the study. The measurements were recorded as follows:

The total tree height of the trees from the ground level to the tip of the tree was measured using Vertex Laser Hypsometer (Haglof, Sweden) and expressed in metre.

Diameter at breast height (DBH) of the trees was measured at 1.37m from the ground level using an electronic digital vernier calliper (Mitutoyo Corporation, Japan) and expressed in centimetre. Basal diameter (BDM) was measured at the collar region using electronic digital vernier calliper and expressed in centimetre.

Volume of the frustum was calculated by Smalian's formula given by Chaturvedi and Khanna (1982). Volume index was calculated as the product of square of DBH and total height and expressed in cm<sup>3</sup>. It was used as a surrogate for total tree volume (Elliott *et al.*, 2002; Luna and Singh, 2007)

Data recorded on the above-mentioned parameters were subjected to analysis of variance (ANOVA) technique for RCBD (Snedecor and Cochran, 1967). The significance of difference among treatment means was tested by 'F' test and the means were compared using Duncan's Multiple Range Test (DMRT). Phenotypic variance, genotypic variance, phenotypic coefficient of variation, genotypic coefficient of variation, broad-sense heritability and genetic advance were estimated for all the characters at age 3. Analysis of variance method was used for estimating the variability parameters.

Data collected on all the parameters from all the provenances were used for genetic divergence analysis. Prior to taking up studies on genetic divergence, using 'V' statistics, which, in turn, uses Wilk's criterion, a simultaneous test of differences between mean values of correlated variables was carried out (Rao, 1948). Wilk's criterion was estimated. The value of 'V' statistic was compared with the tabulated chi-square value for pq degrees of freedom and thus significance was tested.

Mahalanobis' D<sup>2</sup> technique (Mahalanobis, 1928) was used to study the genetic divergence in the international provenances of *C. equisetifolia*. Tocher's method was used to group the various provenances into different clusters (Rao, 1952). The intra and inter cluster relationships were studied after forming the clusters.

## RESULTS AND DISCUSSION

**Mean Performance for Various Characters:** The results obtained due to ANOVA and further Duncan's Multiple Range Test are presented in **Table 1**. Tree height varied from 4.88 m to 8.30 m with a mean and standard deviation of 6.63 m and 0.16 m respectively. The provenance 18586 China ranked first and 17 other provenances were at par with it. 18122 Egypt recorded the minimum value for tree height.

The provenance 18298 Thailand recorded the maximum value for DBH (7.21 cm) and 18137 Kenya the lowest (3028 cm). The mean and SD were 5.04 cm and 0.13 cm respectively. Four other provenances namely, Mixed seedlot India, 18586 China, Palacherla A- India and 18160 Malaysia also registered superior values for DBH. Basal diameter ranged between 4.04 cm and 8.87 cm with a mean and SD of 6.20 cm and 0.13 cm. Five provenances recorded superior values for BDM and the maximum value was exhibited by 18298 Thailand similar to DBH. The minimum value was registered by the provenance 18137 Kenya. When frustum volume was analysed, the provenance 18298 Thailand ranked first (7116.29 cm<sup>3</sup>). Three other provenances were at par with the top ranker. The minimum value was recorded by 18137 Kenya. The mean and standard deviations were 3710.71 cm<sup>3</sup> and 187.23 cm<sup>3</sup> respectively.

The provenance 18298 Thailand again ranked first for volume index and the values varied from 8346.37 cm<sup>3</sup> to 44850.29 cm<sup>3</sup> with a mean and SD of 20050.46 cm<sup>3</sup> and 1439.27 cm<sup>3</sup>. The minimum value was recorded by 18344 Malaysia. Provenances Mixed Seedlot - India and 18586 China were at par with the top ranker.

Among the three primary characters which contribute directly towards volume growth (frustum volume and volume index), DBH showed higher degree of variation than total height in all the provenances. In *Lagerstroemia speciosa*, Jamaludheen *et al.* (1995) observed similar results that height of trees showed less variation than girth. Among the provenances, the best provenance (18298 Thailand) was 223.70 per cent superior than the average of all the provenances when volume index was considered and the difference between the best and the worst provenance (18344 Malaysia) was 537.38 per cent. El-Lakany *et al.* (1990) reported that there were highly significant differences among the provenances of *Casuarina cunninghamiana* and *C. glauca*. The best provenance among the former was 31 per cent taller than the species average and 212 per cent taller than the poorest provenance. Within provenances, the best family was 46 per cent taller than the average of the families and the difference between the best and the worst family of the same provenance was 239 per cent (El-Lakany *et al.*, 1990). Significant differences in growth rate among the provenances of *C. equisetifolia* were also reported by Zhigang and Pengxin (1990).

Table 1. Mean performance of various parameters of *Casuarina equisetifolia* provenances

Provenances	Height (m)	DBH (cm)	BDM (cm)	Frustum Volume (cm <sup>3</sup> )	Volume Index (cm <sup>3</sup> )
17577 PNG	6.06 <sup>a-d</sup>	4.34 <sup>d-f</sup>	5.34 <sup>d-f</sup>	2619.13 <sup>e-f</sup>	11880.25 <sup>g-i</sup>
18008 NT Australia	7.00 <sup>a-d</sup>	4.88 <sup>c-f</sup>	6.00 <sup>c-f</sup>	3442.24 <sup>c-f</sup>	19790.36 <sup>c-e</sup>
18122 Egypt	4.88 <sup>d</sup>	3.84 <sup>e-f</sup>	4.72 <sup>e-f</sup>	2521.58 <sup>e-f</sup>	12360.41 <sup>f-i</sup>
18128 Vietnam	6.70 <sup>a-d</sup>	4.77 <sup>c-f</sup>	5.86 <sup>c-f</sup>	3152.65 <sup>c-f</sup>	16320.15 <sup>d-g</sup>
18134 Kenya	5.99 <sup>a-d</sup>	5.41 <sup>b-e</sup>	6.66 <sup>b-e</sup>	4031.11 <sup>c-f</sup>	18220.26 <sup>c-f</sup>
18135 Kenya	6.31 <sup>a-d</sup>	4.72 <sup>c-f</sup>	5.81 <sup>c-f</sup>	3200.23 <sup>c-f</sup>	16630.39 <sup>d-g</sup>
18137 Kenya	5.50 <sup>c-d</sup>	3.28 <sup>f</sup>	4.04 <sup>f</sup>	1785.22 <sup>f</sup>	9344.27 <sup>i</sup>
18141 Kenya	5.84 <sup>b-d</sup>	4.28 <sup>d-f</sup>	5.26 <sup>d-f</sup>	2685.57 <sup>e-f</sup>	12420.19 <sup>f-i</sup>
18144 Kenya	7.42 <sup>a-c</sup>	5.18 <sup>b-e</sup>	6.38 <sup>b-e</sup>	3682.29 <sup>c-f</sup>	20450.29 <sup>c-e</sup>
18160 Malaysia	6.93 <sup>a-d</sup>	5.75 <sup>a-d</sup>	7.07 <sup>a-d</sup>	4498.35 <sup>b-e</sup>	23180.37 <sup>c</sup>
18267 China	7.08 <sup>a-d</sup>	5.34 <sup>b-e</sup>	6.56 <sup>b-e</sup>	3967.19 <sup>c-f</sup>	22230.59 <sup>c-d</sup>
18297 Thailand	5.48 <sup>c-d</sup>	5.16 <sup>b-e</sup>	6.34 <sup>b-e</sup>	3791.24 <sup>c-f</sup>	15630.47 <sup>e-h</sup>
18298 Thailand	8.24 <sup>a-b</sup>	7.21 <sup>a</sup>	8.87 <sup>a</sup>	7116.29 <sup>a</sup>	44850.29 <sup>a</sup>
18344 Malaysia	5.89 <sup>a-d</sup>	3.62 <sup>e-f</sup>	4.45 <sup>e-f</sup>	1845.59 <sup>f</sup>	8346.37 <sup>i</sup>
18378 QLD	6.46 <sup>a-d</sup>	5.18 <sup>b-e</sup>	6.37 <sup>b-e</sup>	3773.48 <sup>c-f</sup>	18640.14 <sup>c-e</sup>
18153 PNG	6.66 <sup>a-d</sup>	4.66 <sup>c-f</sup>	5.73 <sup>c-f</sup>	3031.24 <sup>d-f</sup>	15490.24 <sup>e-h</sup>
18357 Philippines	6.90 <sup>a-d</sup>	4.91 <sup>c-f</sup>	6.04 <sup>c-f</sup>	3385.18 <sup>c-f</sup>	18420.36 <sup>c-f</sup>
18136 Kenya	5.38 <sup>c-d</sup>	4.11 <sup>d-f</sup>	5.05 <sup>d-f</sup>	2352.27 <sup>e-f</sup>	9968.27 <sup>h-i</sup>
18586 China	8.30 <sup>a</sup>	6.29 <sup>a-c</sup>	7.74 <sup>a-c</sup>	5686.97 <sup>a-c</sup>	39890.19 <sup>a</sup>
18355 Benin	7.45 <sup>a-c</sup>	5.00 <sup>c-f</sup>	6.15 <sup>c-f</sup>	3516.32 <sup>c-f</sup>	19940.11 <sup>c-e</sup>
18268 China	6.70 <sup>a-d</sup>	5.05 <sup>b-f</sup>	6.22 <sup>b-f</sup>	3461.69 <sup>c-f</sup>	17240.37 <sup>c-g</sup>
Palacherla - A India	7.16 <sup>a-d</sup>	6.21 <sup>a-c</sup>	7.63 <sup>a-c</sup>	5340.11 <sup>a-d</sup>	28940.12 <sup>b</sup>
Mixed Seedlot India	8.10 <sup>a-b</sup>	6.80 <sup>a-b</sup>	8.37 <sup>a-b</sup>	6469.28 <sup>a-b</sup>	40980.34 <sup>a</sup>
Mean	6.63	5.04	6.20	3710.71	20050.46
SD	1.71	1.43	1.76	2007.81	15434.42
SEM	0.16	0.13	0.16	187.23	1439.27

\* Means with the same letter in a column do not differ significantly as per Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Variability Parameters: Volume index recorded the maximum GCV followed by frustum volume in all the provenances at 3 years of age (Table 2). Tree height registered the lowest value for GCV and PCV (5.93% and 27.21% respectively). Low GCV for height has been reported in *Bambusa pallida* (Singh, 1993) and *E. tereticornis* (Sundararaju et al., 1995). For all the characters, the PCV was higher than the GCV. This indicated that these parameters were influenced by non-additive gene action (Venkataramanan, 1996; Paramathma et al., 1997; Surendran et al., 2002; Geethanjali et al., 2023).

The proportions of variation in the population that is attributable to genetic differences among individuals can be expressed by heritability values. Heritability values have got great importance in estimating gains that can be obtained from selection programmes (Zobel and Talbert, 1984). With time, space and location, the heritability values vary. For ranking the importance of different traits in cross breeding programmes, heritability values are very useful. For characters that are strongly under genetic

control and have a wide range of variability, the best genetic gains could be obtained (Zobel, 1971; Patel et al., 2022). Among the various characters studied, frustum volume recorded the maximum heritability (0.24) followed by DBH, BDM and volume index (0.20). The lowest value was for tree height (0.05).

Heritability indicates the effectiveness with which the selection of the genotype is based on the phenotype, but fails to convey the genetic progress (Johnson et al., 1955). High heritability estimates therefore, does not necessarily imply greater gain (Ramanujam and Thirumalachar, 1967; Baghyalakshmi et al., 2023). Burton (1951) suggested that the genotypic coefficient of variation together with heritability estimates would give the best results of the genetic advance to be expected from selection. Bagchi and Singh (1994) also proved this concept. Heritability estimates in broad-sense will be reliable if accompanied by high genetic advance as well. In this study, frustum volume and volume index exhibited reasonably higher values for heritability coupled with genetic advance (Table 2).

**Table 2. Genetic parameters of various characters of *Casuarina equisetifolia* provenances**

Characters	GCV	PCV	ECV	Heritability (H <sup>2</sup> )	Genetic Advance
Height	5.93	27.21	26.55	0.05	2.66
DBH	13.25	29.64	26.52	0.20	12.20
CDM	13.24	29.63	26.51	0.20	12.19
FV	26.68	54.22	47.20	0.24	27.04
D2H	34.44	77.15	69.04	0.20	31.67

According to Zobel and Talbert (1984), even in large experiments with many families, heritabilities are not estimated without error. All the heritability values should be thought of as being figures that give a general idea of strength of inheritance for example, a heritability of 0.15 should not be thought of as being much different than a heritability of 0.20. The main value of the heritability concept to the tree breeder is to indicate the general strength of genetic control and the best approach for use in tree improvement.

Genetic Divergence: Genetic divergence analysis is essential to estimate the genetic diversity existing among the various provenances / genotypes of a species. For identifying the genotypes for hybridization purposes divergence analysis is essential (Singh and Chaudhary, 1985). Among the various methods available, Mahalanobis' D<sup>2</sup> analysis technique is the most appropriate measure of multivariate relationships when the data are normally distributed and homocedastic. Also known as generalized distance method, it can be useful even if the above assumption is not quite true.

Results of the genetic divergence studies are presented in **Tables 3 to 7**. Mahalanobis' D<sup>2</sup> statistics followed by Tocher's clustering method grouped the 23 provenances of *C. equisetifolia* into 9 clusters with cluster strength varying from one (clusters 8 and 9) to eight (cluster 4). Cluster 7 contained three provenances and the remaining five clusters had two provenances each.

Average intra and inter cluster distances: The average intra and inter cluster D<sup>2</sup> and D values for the 9 clusters obtained are presented in **Tables 4 and 5**. The largest intra-cluster distance was registered in cluster 7 (1.84) followed by cluster 4 (1.58). Cluster 1 recorded the smallest intra-cluster distance (0.28) followed by clusters 2 and 3 (0.43). Intra cluster distance for cluster 8 and 9 was zero, since they included only one provenance each. Based on the inter-cluster distances it was evident that the maximum divergence existed between clusters 6 and 9 (2.95). The minimum inter-cluster distance was observed between clusters 2 and 5 (0.77). Higher values for inter-cluster distance were also recorded between clusters 6 and 7 (2.90) and clusters 7 and 8 (2.47) when compared to

**Table 3. Clustering of provenances of *Casuarina equisetifolia***

Cluster No	Provenances
Cluster 1	18357 Philippines 18136 Kenya
Cluster 2	18008 NT Australia 18144 Kenya
Cluster 3	18160 Malaysia 18268 China
Cluster 4	17577 PNG 18122 Egypt 18128 Vietnam 18134 Kenya 18135 Kenya 18137 Kenya 18267 China 18153 PNG
Cluster 5	18297 Thailand Palacherla - A India
Cluster 6	18141 Kenya 18378 QLD
Cluster 7	18298 Thailand 18344 Malaysia Mixed Seedlot India
Cluster 8	18586 China
Cluster 9	18355 Benin

**Table 4. Inter and intra cluster D square values**

	1	2	3	4	5	6	7	8	9
1	0.08	0.30	0.67	1.69	0.32	2.53	4.78	1.70	4.39
2		0.18	1.31	1.61	0.59	2.23	4.94	1.07	5.20
3			0.19	2.61	1.16	2.27	5.05	3.12	5.41
4				2.50	1.53	4.28	3.92	2.54	4.29
5					0.25	3.94	3.81	1.53	3.66
6						0.94	8.41	4.07	8.71
7							3.38	6.11	4.93
8								0.00	5.80
9									0.00

**Table 5. Inter and intra cluster distances**

	1	2	3	4	5	6	7	8	9
1	0.28	0.55	0.82	1.30	0.56	1.59	2.19	1.30	2.10
2		0.43	1.14	1.27	0.77	1.49	2.22	1.03	2.28
3			0.43	1.62	1.08	1.51	2.25	1.77	2.33
4				1.58	1.24	2.07	1.98	1.59	2.07
5					0.50	1.98	1.95	1.24	1.91
6						0.97	2.90	2.02	2.95
7							1.84	2.47	2.22
8								0.00	2.41
9									0.00

other pairs. Cluster means of characters: Cluster means computed for all the five characters are given in **Table 6**. Cluster 9 which contained one provenance, 18355 Benin registered the maximum mean values for all the traits including tree height, DBH, BDM, frustum volume and volume index. Cluster 8 exhibited the minimum values for total height (5.38), CDM (5.19). Minimum values for DBH (3.45), BDM (4.25), frustum volume (1814.92) and volume index (8845.12) were noticed in cluster 6. Clusters 7, 5 and 4 also registered higher mean values for frustum volume and volume index.

Contribution of characters towards genetic divergence: Relative contribution of each character towards genetic divergence is presented in **Table 7**. Volume index

contributed the maximum towards genetic divergence (43.48%) followed by frustum volume (22.53%), and DBH (18.58%). The minimum percentage contribution towards genetic divergence was registered by tree height (4.35%).

Choice of the particular cluster from which genotypes are to be used as parents, selection of a particular genotype from the selected clusters and the relative contribution of characters to total divergence are the three essential points which need to be considered while selecting the genotypes (Singh and Chaudhary, 1985). The results obtained in this study suggest that selected provenances included in clusters 6,7,8 and 9 in general can be used for hybridization programmes to create variability and exploit hybrid vigour. Therefore, the suggested provenances for

**Table 6. Cluster means**

Clusters	Height	DBH	CDM	Frustum Volume	Volume Index
1	6.78	4.79	5.89	3207.70	16958.94
2	5.95	4.31	5.30	2651.55	12152.05
3	7.44	5.09	6.26	3599.10	20192.87
4	6.34	4.96	6.10	3645.22	19269.02
5	6.89	5.20	6.39	3714.03	19734.75
6	5.69	3.45	4.25	1814.92	8845.12
7	6.96	6.19	7.62	5415.49	29806.96
8	5.38	4.11	5.05	2352.17	9967.51
9	8.30	6.29	7.74	5686.31	39886.75



Table 7. Contribution of each character to divergence

Character	No of First Rank	% Contribution
Tree Height	11	4.35
DBH	47	18.58
BDM	28	11.07
Frustum Volume	57	22.53
Volume Index	110	43.48
Total	253	100.00

future breeding programmes are 18141 Kenya, 18378 QLD, 18298 Thailand, 18344 Malaysia, Mixed Seedlot India, 18586 China and 18355 Benin.

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