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GENETIC DIVERGENCE FOR GROWTH AND WOOD PARAMETERS IN DIFFERENT CLONES OF *CASUARINA EQUISETIFOLIA*

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Forty-six *Casuarina equisetifolia* clones were evaluated for growth and wood parameters to study their genetic divergence. The clones were grouped into seven clusters using Mahalanobis D^2 analysis. Vessel frequency contributed maximum (51.50%) towards divergence followed by specific gravity (16.23%). Maximum inter-cluster distance was observed between clusters 3 and 5 (14.58) followed by clusters 5 and 7 (12.098). Intra-cluster distance was maximum in cluster 5 (9.225) followed by clusters 6 (8.991), 7 (8.068) and 1 (7.700). Highest value of heritability, genetic advance and genetic gain were obtained for specific gravity, fibre length and volume respectively. Both phenotypic coefficients of variation (PCV) and environmental coefficients of variation (ECV) were highest for vessel frequency, while genotypic coefficients of variation (GCV) was highest for volume. ECV was observed to be higher than GCV for all the traits showing the influence of environmental factors on these traits. The results on genetic divergence had potential for immediate application in the establishment of clonal seed orchards. Information on genetic divergence provides an opportunity for hybridisation among the genotypes and obtaining quality seeds with high vigour.

Keywords: Genetic divergence, fibre morphology, vessel morphology, specific gravity, genotypic variation, heritability, phenotypic coefficients of variation, genotypic coefficients of variation

INTRODUCTION

Casuarina equisetifolia, a fast growing and multipurpose tree species, is indigenous to the tropics and subtropics of South-East Asia and western Pacific regions, including northern Australia (Ogata et al. 2008). It is considered an important multipurpose tree on account of its various uses such as in nitrogen fixing, wind breaks, control of soil erosion, fuel wood, poles and production of pulp and paper. Casuarinas contribute to a considerable area of plantation forests, both in the tropics and subtropics. Among casuarinas, *C. equisetifolia* is one of the most extensively planted species in India. The demand for *C. equisetifolia* wood had been increasing dramatically as it is an excellent raw material in pulp and paper industries and preferred as poles and scaffoldings (Kumar & Gurumurthi 1996). Realising the socio-economic importance of this species, the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore started a tree improvement programme of *Casuarina equisetifolia* in the early 1990's. The breeding programme of *C. equisetifolia* commenced in 1996 with assistance from the Forest Tree Improvement Project and Planting Stock Improvement Programme of the Forestry Research Education and Extension Project, which was funded by the World Bank. A large number of seed production populations have been established, yielding genetically improved seeds. IFGTB has successfully produced commercially useful clones of this species with improved productivity and these were investigated in the present study.

Genetic divergence studies are an effective tool for establishing seed orchards for forest tree species with diverse parents. This way, improved seeds harvested are highly effective and economical because diverse parents have equal

opportunities for hybridisation and production of quality seeds (Kumar & Gurumurthi 2000). This technique has been effectively applied to distinct populations of *Eucalyptus camaldulensis* and *Acacia nilotica* (Burley et al. 1971, Bagchi 1992). Divergence studies analyse the degree of diversification and relative proportion of each component character to the total divergence, which measures the forces of differentiation at intra- and inter-cluster levels (Pande et al. 2013). Assessing all available genetic variations or divergence within/between species and delineation of best genotype matching to the site for increased productivity is of prime important in any tree improvement programme (Sharma & Bakshi 2014). Better understanding of genetic variation among species helps to produce superior hybrids/clones. Hybrids between individuals of diverse origin display greater heterosis than those between closely related individuals. Screening and evaluation of genetically divergent clones or hybrids can be used for further tree improvement programmes. The present work was formulated to assess the genetic divergence in growth and wood physical and anatomical parameters between 5-year-old *C. equisetifolia* clones which could be further exploited for future breeding and improvement programmes of this species.

MATERIALS AND METHODS

Selection of samples

Clones were developed as the part of the tree improvement programme of IFGTB. In order to widen the genetic base of clones used in farm forestry, IFGTB conducted clonal testing with a large collection of clones. The clones were selected from the genetic gain trial established at Puducherry in 2003 using bulk seeds obtained from the first generation seedling seed orchard at Sadivayal, Coimbatore. A total of 87 clones and 3 seed lots were selected. The clones were selected based on individual tree superiority for height, diameter at breast height (DBH) and straightness of stem through index selection method. These clones were tested in three locations in Tamil Nadu, namely, (1) Mayiladumparai, near Kulithalai (10° 56' N, 78° 25' E) (2) Moorthypuram, near Karur (10°57' N, 78° 04' E) and (3) Sirugramam, near Cuddalore (11° 44' N, 79° 46' E). The spacing adopted was 3 m × 5 m with three ramets (trees) per clone. A total of 90 assertions were tested in these three locations. Forty-six assertions tested in Karur were selected for the present study. The clones were field planted in a completely randomised design with eight replications.

Preparation of wood samples

Billets, each 1 m long, were cut from the basal position of three ramets, each selected randomly from the clones. Transverse discs each 6 cm thick, were collected from the base, middle, and top of each billet. For studies of wood physical and anatomical properties, discs were cut into two transverse halves. One half was used for estimation of wood specific gravity and the other half was used for studying anatomical properties. For specific gravity measurements five wood blocks of dimensions 2 cm × 2 cm × 2 cm were taken from the discs.

Determination of physical and anatomical properties

Specific gravity was estimated based on oven dry weight divided by oven dry volume using specific gravity module attached to a precision electronic balance. For fibre morphological studies, wood shavings were taken from the halved transverse disc and macerated using Jeffrey's method (Sass 1951). Jeffrey's solution was prepared by mixing equal volumes of 10% potassium dichromate and 10% nitric acid. Microscopic examination and quantification of macerated fibres were conducted using an image analyser. For vessel morphology study, sections were taken from specimens of size less than 1 cm^3 using microtomy. Sections were then stained using saffranin for 5 min. Excess stain was removed by washing the sections successively in 70, 90 and 95% ethanol solution. Thin sections were further dehydrated using acetone and kept in xylene for 2 hours to give them sufficient rigidity. Sections were permanently mounted on microscope slides with coverslips using DPX.

Statistical analysis

The data used for this study were subjected to analysis of variance (ANOVA) in order to quantify the variation existing between clones for various recorded parameters. Genotypic and phenotypic

components of variance of all these characters were calculated from analysis of variance table as described by Burton (1952) and Pearson's correlation coefficients of characters were calculated.

Estimation of genetic parameters and genetic divergence

Phenotypic and genotypic variance values were estimated according to Johnson et al. (1955) using the formulae given below:

$$\text{Genotypic variance } (V_g) = (M_t - M_e)/r$$

where M_t = mean sum of square of treatment, M_e = mean sum of square of error, and r = block replicates. M_t and M_e were obtained from ANOVA table. M_e was taken as environmental variance (V_e), i.e. $V_e = M_e$.

$$\text{Phenotypic variance } (V_p) = V_g + V_e$$

Phenotypic coefficient of variability, PCV (%)=

$$\sqrt{\frac{\text{Phenotypic variance}}{\text{Mean}}} \times 100$$

Genotypic coefficient of variability, GCV (%) =

$$\sqrt{\frac{\text{Genotypic variance}}{\text{Mean}}} \times 100$$

Broad sense heritability (H^2) was calculated according to Lush (1937).

$$H^2 \text{ percentage} = V_g / V_p \times 100$$

Genetic advance (GA) was worked out after Johnson et al. (1955):

$$GA = ½(V_g / V_p) \times K$$

where, K = 2.06, a selection differential at 5% selection intensity. Genetic advance was calculated as a percentage of mean = (GA/grand mean) × 100. Genetic gain expected in per cent of mean was calculated using the formula given Johnson et al. (1955).

Genetic gain = (GA/N) × 100

where, N = total mean of the clones.

Mahalanobis D^2 statistics (Mahalanobis 1936) was used for the calculation of genetic divergence between clones using GENRES software. Grouping of the clones into various clusters was done based on growth, physical and morphological parameters using Mahalanobis D^2 statistics.

RESULTS AND DISCUSSION

Genetic and environmental variations are very important and effective for breeders to develop superior trees/clones in any tree improvement programme. Genetic variability/divergence plays an important role in any of the breeding/improvement programme since hybrids between individuals of diverse origin display greater heterosis than those between closely related individuals. The present study was carried out to understand genetic variability/divergence among 46 clones of *Casuarina equisetifolia* grown in trials established in Karur district, Tamil Nadu, south India. On the basis of genetic divergence, clones were grouped into 7 clusters (Table 1). The cluster behaviour and genetic distance between them would help to maintain genetically diverse population of high yielding clones (Kumar & Gurumurthi 2000). Maximum number of clones were included in cluster 6 (27 clones) followed by clusters 1 (6 clones) and 7 (4 clones), whereas the minimum for clusters 2, 3 and 4 (2 clones each). Even though the clones were from different geographical regions, majority of the clones clustered together to form one large cluster (27 clones). This may be due to similarity in growth as well as physical and anatomical parameters of clones resulting from site and environmental conditions. In a genetic divergence study based on growth parameters of *C. equisetifolia,* Kumar and Gurumurthi (2000) grouped 42 clones into 12 clusters. Pande et al. (2013) grouped 28 populations into six clusters based on wood and growth parameters of *Leucaena leucocephala.*

Mean values of traits for all the clusters were also measured. Usually, one or two clusters would be superior from all the other clusters considering the highest and lowest values for majority of the traits. In this case, no such cluster was derived; instead the highest value of each trait was distributed in different clusters (Table 2). Maximum mean height, DBH and volume were obtained for clusters 1, 2 and 4 respectively. Specific gravity was highest for cluster 3 while fibre length and fibre wall thickness for cluster 1 and fibre diameter for cluster 6. Vessel length, vessel diameter and vessel frequency were maximum

Table 1 Constituents of different clusters formed based on Mahalanobis D^2 statistics using growth, physical and morphological parameters for *Casuarina equisetifolia*

Cluster no	No. of clones	Clones
1	6	TNPP 1 (M), CE 2003/4 (M), TNRM 8 (F), TNPP 2 (F), CE 2002/1 (F), TNRM 5 (X)
2	2	JKCE 6 (F), Seedling origin 1 (X)
3	2	JKCE 8 (F), TCR 120102 (F)
4	2	TNCS1 (M), CE 2002/2 (X)
5	3	TNVM 2 (M), CE 2003/3 (M), CE 268 (F)
6	27	CE 128 (M), CE 219 (M), TNPV 4 (M), TNVM 3 (M), CE 276 (M), APKKD 6 (M), CH 0905 (M), APSKLM 25 (M), CE 332 (M), CE 2003/5 (F), CE 303 (F), TNIPT 16 (F), CE 100 (F), TN 111 (F), CH 2803 (F), APVSP 14 (F), TCR 060101 (F), CE 220 (F), CE 80 (F), CE 243 (F), CE 9 (F), CE 281 (F), CE 83 (X), CH 2602 (X), CE 329 (X), CE 327 (X), CE 224 (X)
7	4	CH 3001 (M), TCR 120203 (F) Seedling origin 2 (X), Seedling origin 3 (X)

M = male, F = female and X = monoecious

Table 2 Mean values of growth parameters for clusters of *C. equisetifolia*

Cluster no.	Height (m)	DBH (cm)	Volume (cm^3)	SG (OD)	FL (µm)	FD (µm)	FWT (µm)	VL (µm)	VD (µm)	VF (no of vessels per mm^2)
1	11.135	7.032	57562.61	0.77	1671.19	27.85	8.54	543.25	162.22	9.41
2	10.642	7.323	58709.87	0.78	1626.14	27.64	8.20	539.42	174.52	7.52
3	11.414	6.739	52802.50	0.83	1620.03	26.81	8.39	513.69	187.59	6.80
4	10.956	7.248	59413.40	0.78	1607.52	27.25	8.46	582.52	172.71	8.74
5	10.924	6.792	52145.95	0.76	1533.49	27.73	8.19	581.43	148.54	11.74
6	10.658	6.805	52608.07	0.78	1622.09	29.32	8.44	539.47	172.57	9.33
7	10.726	6.824	51738.72	0.70	1522.44	27.14	8.40	510.95	178.65	8.13

DBH = diameter at breast height, SD (OD) = specific gravity, FL = fibre length, FD = fibre diameter, FWT = fibre wall thickness, VL = vessel length, VD = vessel diameter, VF = vessel frequency

for clusters 4, 3 and 5 respectively. Cluster 7 showed minimum values for volume, specific gravity, fibre length and vessel length whereas the minimum values for DBH, fibre diameter and vessel frequency occurred in cluster 3. Fibre wall thickness and vessel diameter was observed in cluster 5 and height in cluster 2. Among the different traits studied, specific gravity could be considered as the most important parameter for selection of clusters because of its high heritability. Hence, cluster 3 was preferred over the rest of the clusters. Even though volume was higher in cluster 4, its specific gravity and tree height were low compared with cluster 3. Fibre length is important for any pulpwood species. All clusters in this study showed almost uniform fibre length except for clusters 5 and 7. Vessel parameters of clusters might be considered for the selection of trees for ecological adaptation.

The higher values for different parameters observed in different clusters may be due to highly divergent nature of clusters. This was evident in the higher inter cluster distances between clusters. The higher the distance, the greater the divergence among clusters. The inter- and intra-cluster distances were calculated using Mahalanobis D^2 analysis and thereby genetic divergence among clusters could be analysed. Both growth and physico-anatomical parameters were used for analysis. When cluster distances were examined, it was found that cluster 7 was highly divergent from all the other clusters. Genetic divergence was moderately high when cluster distances between the rest of the combinations were studied. Maximum inter -cluster distance was observed between cluster 3 and cluster 5 (14.581) followed by clusters 5 and 7 (12.098), and clusters 3 and 7 (11.695).

The inter-cluster distance was minimum between clusters 2 and 4 (3.364) followed by clusters 2 and 3 (5.732), and clusters 1 and 2 (6.864). Intra-cluster distance showed higher values for majority of the clusters such as clusters 5 (9.225), 6 (8.991), 7 (8.068) and 1 (7.700). As the higher values of intra-cluster distance could be attributed to higher genetic divergence of clones within the cluster, the clones from any one of these clusters such as clusters 1, 5, 6 and 7 could be selected for tree improvement programme through hybridistion. Inter- and intra-cluster distances calculated were presented in Table 3. Hybridisation between the populations selected from diverse clusters is expected to express higher heterosis and produce desirable recombinants and transgressive segregants (Pande et al 2013). It is also important to obtain immediate gain of high diversity by selecting these populations for establishment of seed orchards.

Divergence studies also analyse the degree of diversification and relative contribution of each component character to the total divergence (Table 4). In the present study, vessel frequency contributed maximum (51.50%) towards divergence followed by specific gravity (16.23%), and vessel diameter (11.11%). Since these parameters contributed substantially towards total divergence they could well be used as desirable traits for selection. All other parameters had little contribution to divergence. The differences in the contributing factors for genetic divergence could be attributed to differences between clones studied, which might be due to environmental conditions at the locations of the clones and their associated interaction.

As almost all the characters showed moderately high phenotypic variation, greater improvement could be expected in the selection of genetic parameters. Among these parameters maximum phenotypic coefficient of variation was observed for vessel frequency (46.02) followed by volume (39.74), vessel length (26.22) and fibre wall thickness (23.40) (Table 5). However, compared with phenotypic coefficient of variation,

Table 3 Intra- and inter-distances of clusters classified based on parameters of *C. equisetifolia*

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	7.700	6.864	10.065	5.958	9.300	8.435	9.681
Cluster 2		2.381	5.732	3.364	11.215	7.435	7.912
Cluster 3			2.557	7.002	14.581	9.469	11.695
Cluster 4				3.028	9.363	6.905	7.954
Cluster 5					9.225	10.898	12.098
Cluster 6						8.991	10.623
Cluster 7							8.068

Table 4 Percentage contribution of each character towards divergence of *C. equisetifolia*

Character	% contribution
Tree height	0
Diameter at breast height	0.29
Volume	0.29
Specific gravity	16.23
Fibre length	4.92
Fibre diameter	7.15
Fibre cell wall thickness	0
Vessel length	8.50
Vessel diameter	11.11
Vessel frequency	51.50
Total	100

Table 5 Estimation of heritability percentage, genetic advance (GA), genetic gain (GG), genotypic coefficients of variation (GCV), phenotypic coefficients of variation (PCV), and environmental coefficients of variation in (ECV) of various traits of *C. equisetifolia*

Character	Growth character (%)					
	Heritability	GA	GG	GCV	PCV	ECV
Tree height	14.199	0.366	3.393	4.372	11.602	10.747
DBH	32.766	0.795	11.564	9.807	17.133	14.049
Volume	26.182	0.012	21.433	20.333	39.738	34.142
SG	47.788	0.066	9.040	6.348	9.183	6.636
FL	18.831	76.400	5.260	5.884	13.560	12.217
FD	17.731	1.469	5.831	6.722	15.964	14.479
FCWT	11.331	0.424	5.463	7.878	23.403	22.037
VL	11.376	27.050	6.144	8.843	26.219	24.683
VD	16.819	8.670	5.714	6.764	16.493	15.042
VF	13.618	0.979	12.911	16.984	46.024	42.776

DBH = diameter at breast height, SG = specific gravity, FL-fibre length, FD = fibre diameter, FCWT = fibre cell wall thickness, VL = vessel length, VD = vessel diameter, VF = vessel frequency

genotypic coefficient of variation were lower in magnitude which indicated a larger influence of environment on these characters. Maximum genotypic coefficient of variation was obtained for volume (20.33) followed by vessel frequency (16.98) and DBH (9.81). A large difference in phenotypic coefficient of variation and genotypic coefficient of variation was observed in wood properties of hybrid poplar clones (Huda et al. 2014). Environmental coefficient of variation for all traits were higher than their respective genotypic coefficient of variation and this reflected significant influence of environment on the expression of different traits. For specific gravity, genotypic coefficient of variation and environmental coefficient of variation were almost the same and there was considerable inter-genotypic variation existing for further genetic improvement. Pande et al. (2013) reported higher environmental coefficient of variation for all the traits in *L. leucocephala* except specific gravity and DBH than their corresponding genotypic coefficient of variation.

Heritability is defined as the degree to which a character is influenced by heredity compared with the environment. Higher heritability values indicate that the characters under study are less influenced by environment in their expression and the scope of genetic improvement of these characters through selection is large. In the present study, the estimation of broad sense heritability for various characters showed low to moderate heritability, maximum observed for specific gravity (47.79%) followed by DBH (32.77%) and volume (26.18%). Minimum heritability occurred in vessel length (11.38%) and vessel frequency (13.62). Heritability of specific gravity obtained in the present study (47.79%) is within the reported range for heritability of most of the hardwood species. High heritability in conjunction with high genotypic coefficient of variation is preferable for practicing selection of elite genotypes from diverse genetic population (Hanson et al. 1956). Since specific gravity, DBH, volume and fibre length had moderate heritability and genotypic coefficient of variation, these characters could be considered for future tree improvement programme.

Broad sense heritability estimates could be reliable if accompanied by high genetic advance (Burton & Devane 1953). Maximum genetic advance and genetic gain were obtained for fibre length (76.400) and volume (21.433) respectively and minimum for volume (0.012) and height (3.393) respectively. High heritability coupled with moderate genetic advance and genetic gain were exhibited by volume, DBH, specific gravity, vessel frequency and fibre length which signifies moderate amount of heritable additive genetic component that can be exploited for further selection and improvement of this species. Subramanian et al. (1995) reported that height, clear bole height, girth, diameter and basal area showed moderate heritability and genetic advance in *Eucalyptus grandis*. Pande et al. (2013) also found high heritability coupled with moderate level of genetic advance and genetic gain for girth at breast height, specific

gravity and height in *L. leucocephala*. The rest of the characters in this study showed moderate/low heritability with low genetic advance, indicating the presence of non-additive gene action.

CONCLUSIONS

Studies of genetic diversity as well as genetic distance among large number of intensively selected clones in the clone bank/trails would help to maintain genetically diverse population of high yielding clones. Results from this study demonstrated that high genetic variability is present among *C. equisetifolia* clones for important growth, physical and anatomical traits, which indicate their high potential for effective tree improvement programme and/or for further manipulation of the genetic resources. Genetic distance measured through Mahalanobis D^2 analysis demonstrated the genetic variability between different clusters. Clones present in clusters which had high mean values of desired traits such as tree height, DBH, volume, specific gravity and fibre length could be selected for future tree improvement programmes. Only fibre length had high heritability and high genetic advance. High heritability coupled with moderate genetic advance and/or genetic gain were exhibited by tree height, DBH, volume, specific gravity. This signified that these traits contained good amounts of genetic divergence and heritable additive genetic component could be exploited for further selection and improvement of this species. Ecological significance of clones was demonstrated through vessel characteristics.

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