Genetic Divergence Studies on Clonal Performance of *Casuarina Equisetifolia*

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Abstract

High yielding clones of *Casuarina equisetifolia* were screened and at the age of 12 months genetic divergence among the clones was estimated. On the basis of cluster distance, 42 clones were grouped into 12 clusters, and inter and intra-cluster distance calculated. Mean cluster values showed significant variation among the clusters for all the traits. Cluster 2 had the fastest growing male clone while cluster 8 had the fastest growing female clone. A hybridization model on the clonal material is proposed based on the study.

Key words: Casuarina equisetifolia, clones, variability, genetic divergence, hybridization.

Introduction

The potential of multipurpose tree species in enhancing the diversity, sustainability and productivity of marginal ecosystem has received increased attention in recent years. Casuarinas contribute to a considerable area of plantation forests, both in tropics and subtropics, and are the important tree species for plantation forestry. In India and many other countries, Casuarinas are planted and harvested at 5 to 10 years rotation. In *Casuarina* plantations, the organic matter returns to soil in the form of foliage is relatively high. Casuarinas are the excellent soil reformers and increase soil fertility through nitrogen fixation (DOMMERGUES *et al.,* 1990). Casuarinas are among the most important tree species for production of high yielding wood biomass for fibre, rayon and energy needs (HEGDE, 1993).

The demand of *C. equisetifolia* wood has been increasing dramatically, as it is an excellent raw material in paper and pulp industries and preferred as poles and scaffoldings (ASHOK KUMAR *et al.,* 1996). In China, *C. equisetifolia* is found to be the most suitable planting stock in sandy soils and preferred over other *Casuarina* species (EL-LAKANY *et al.,* 1990). Thus, planting in newer areas with superior performers is vital for increasing the productivity. The fact that the yield variation per tree ranges from 4 kg to 35 kg in a five year old plantation (ASHOK KUMAR, 1996) indicates the need to identify superior performers and introduce in plantation forestry programme. Hence, research needs to be directed to improve the genetic potential of *Casuarina.* Genetic diversity plays an important role in any of the breeding/improvement programme since hybrids between the individuals of diverse origin display greater heterosis than those between closely related individuals. Genetically divergent clones are to be screened and established in the clonal seed orchards for further improvement. The technique has strongly been used to exploit hetrosis by selecting divergent parents, and mating them either artificially or naturally. Thus, it provides an opportunity in selecting divergent parents to use in hybridization and design clonal seed orchards accordingly.

With this background, present study was taken up to estimate genetic divergence by using WARD's hierarchical method of grouping (WARD, 1963a and b). This technique of clustering involves measurements of the forces of differentiation at two levels namely inter and intra, and enables selection of genetically divergent parents for tree improvement/hybridization programme.

Materials and Methods

1. Selection of trees

Five hundred and five phenotypically superior trees of *C. equisetifolia* were selected initially from two different plantation sites, viz. Chengalpet (longitude 80°11'E, latitude 13°00'N) and Chidambaram (longitude 71°11'E, latitude 11°54'N) in the coastal belt of Tamil Nadu, by dividing each of these plantations into 50 grids to minimize soil heterogeneity. In each grid, trees with good height, diameter at breast height (DBH), straight and clear main bole, self pruning capacity and with no incidence of disease and pests were selected, and a selection index based on these characters was constructed.

The index for each character was calculated by multiplying phenotypic value with correlation coefficient of the trait with main bole volume. The total selection was calculated by adding all six values for each selected tree. By this method, 24 candidate trees were finally selected from Chengalpet and 31 trees from Chidambaram (ASHOK KUMAR, 1996).

2. Vegetative propagation

To establish the clone bank, hedge orchard and location trial, the cladodes of selected trees were propagated through rooting in mist and mistless systems of propagation at the Institute of Forest Genetics and Tree Breeding, Coimbatore (ASHOK KUMAR, 1996).

3. Estimation of genetic divergence

The clones were field planted in a completely randomized design (CRD) with eight replications. Genetic divergence of different clones, and group distance based on observations, taken at the age of 12 months, were measured by WARD's method of grouping (WARD, 1963a and b).

Hierarchical groups

In the present study, the grouping process started by selecting two of 42 clones which, when united, reduced by one in number of subsets i.e. n-1 (41). Again, n-1 resulted subsets were observed to determine whether a third subset of clone unites with the pair. If not, another pairing was made in order to secure the optimal value of the objective function for n-2 groups. This procedure was repeated until all the clones were grouped. In this method, the reduction of subsets is systematic (n, n-1,.....,1). Thus, the process is termed as "hierarchical groups or clusters".

Clustering

The hierarchical grouping develops a relationship between clones that are maximally similar for specified characteristics. Each of the clones is genetically different from another. However, the extent of difference might vary to a certain degree. Thus, during clustering number of clusters may vary from one

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to the total number of clones used. To optimize the number of cluster, a cut off line was derived on the WARD's minimum variance dendrogram that was close to medium value. Based on this information number of clusters were developed.

Relationship of clusters

Based on inter cluster distances, the clusters were classified into closely, moderately and highly divergent groups as follows:

Cluster relationship	Cluster distance
Closely divergent (C)	<2.00
Moderately divergent (M)	2.00 – 4.00
Highly divergent (H)	> 4.00

Results and Discussion

In the present study, 42 clones were grouped into 12 clusters. The details of genetic distance and variance between the clones and the clusters are presented in WARD's minimum variance dendrogram (*Fig. 1*). The constituents of different clusters are presented in *table 1*. Cluster 6 was the largest cluster consisting of 9 clones whereas four clusters viz. 2, 5, 9 and 12 consisted of one clone each. Cluster 2 had the fastest growing male clone (CHCE890903) and cluster 8 had the best performing female clone (CHCE 892703). Cluster 2 and 12 were found to be either highly or moderately divergent from all other clusters. Detailed divergence relationship matrix for 12 clusters is presented in *table 2*.

The mean values for different clusters are presented in *table 3*. Mean cluster values showed significant variation among the clusters for all the traits. Cluster 2 had the highest values for all three traits viz. plant height (577 cm), diameter at ground level (DGL, 5.54 cm) and DBH (4.50 cm) followed by cluster 1. Cluster 11 had minimum mean DGL (2.40 cm) and DBH (1.20 cm) values whereas, cluster 12 had minimum plant height (217 cm).

Intra and inter cluster distance of different clusters are presented in *table 2*. The maximum inter cluster distance was recorded between cluster 11 and 2 (8.45) followed by 2 and 12 (6.72), 1 and 11 (6.42) and 2 and 3 (6.32). Whereas minimum inter cluster distance was recorded between cluster 6 and 4 (0.85) and 6 and 7 (0.92). The intra cluster distance ranged from 0.00 (cluster 2, 5, 9 and 12) to 1.24 (cluster 1).

The maximum heterosis occurs at an optimal or intermediate level of divergence (SINGH, 1993). In addition to aiding the selection of divergent parents for hybridization and establishment of clonal seed orchard, this technique measures the degree of diversification and determines the relative proportion of each component character to the total divergence. The genotypes grouped together are less divergent than the ones placed in different clusters. Genetic divergence studies are also an effective tool for establishing seed orchards of divergent genotypes. Obtaining information on genetic distance between the genotype helps in developing planting design, such that it can facilitate equal opportunity for hybridization among the genotypes and obtaining quality seed with high vigour.

Hybridization requires selection of both male and female parents from two divergent clusters. Based on data it is inferred that male clones of cluster 1 and 2 could be used in a number of combinations as they are highly divergent from the other male clones. Though cluster 1 had two male clones, the intra clonal distance was very high (1.24), hence both the clones are adequately divergent for hybridization. To obtain immediate gain of high diversity, these clones can be used for establishing a clonal seed orchard.

Genetic divergence studies have also been extensively carried out to determine the genetic divergence in the provenance (BURLEY *et al.,* 1971; ANDREW, 1973; BAGCHI, 1992) and progeny trials (SINGH and CHAUDHARY, 1992). BHATT (1973) selected parents for hybridization programme using four different techniques and compared the performance of hybrids, and found that the genetic divergence analysis was the most effective tool to identify the parents for hybridization. LEGIONNET *et al.* (1999) found that susceptibility of foliar rust in *Populus nigra* was best represented on second axis. However, literature on divergence analysis among the clones is non existent.

Clonal strategy is alleged to offer narrow genetic base, yet vegetative propagation has strongly been recommended (CAM-

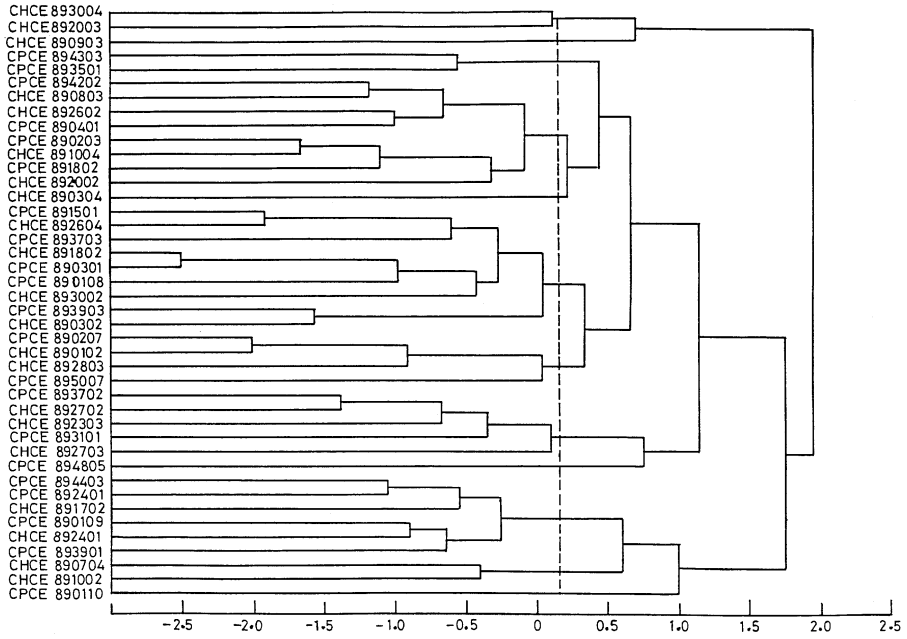

Fig. 1. – WARD's minimum variance dendrogram.

Table 1. – Constituents of different cluster.

Cluster Number	No. of clones in the cluster	Clone number and sex of the clone	
1	2	1. CHCE893004 (M)	2. CHCE892003 (M)
2	1	1. CHCE890903 (M)	
3	2	1. CPCE894305 (M)	2. CPCE893501 (F)
4	8	1. CPCE894202 (M) 3. CHCE892602 (F) 5. CPCE890203 (M) 7. CPCE891802 (F)	2. CHCE890803 (M) 4. CPCE890401 (M) 6. CHCE891004 (M) 8. CHCE892002 (M)
5	1	1. CHCE890304 (M)	
6	9	1. CPCE891501 (M) 3. CPCE893703 (F) 5. CPCE890301 (F) 7. CHCE893002 (F) 9. CHCE890302 (M)	2. CHCE892604 (M) 4. CHCE891802 (F) 6. CPCE890108 (M) 8. CPCE893903 (M)
7	4	1. CPCE890207 (M) 3. CHCE892803 (F)	2. CHCE890102 (F) 4. CPCE895007 (F)
8	5	1. CPCE893702 (F) 3. CHCE892303 (M) 5. CHCE892703 (F)	2. CHCE892702 (M) 4. CPCE893101 (M)
9	1	1. CPCE894805 (M)	
10	6	1. CPCE894403 (F) 3. CHCE891702 (M) 5. CHCE892401 (F)	2. CPCE892401 (F) 4. CPCE890109 (F) 6. CPCE893901 (M)
11	2	1. CHCE890704 (F)	2. CHCE891002 (F)
12	1	1. CPCE890110 (M)	

Figures in parenthesis indicate sex of the clones.

Table 3. – Cluster means for 12 different clusters of *Casuarina equisetifolia* at the age of 12 months.

Cluster number	Cluster means		
	Plant height (cm)	DGL (cm)	DBH (cm)
1	533	4.71	3.58
2	577	5.54	4.51
3	439	2.94	1.91
4	391	3.35	2.20
5	396	3.54	1.61
6	436	3.48	2.23
7	413	3.73	2.54
8	505	3.73	2.53
9	407	3.72	3.46
10	340	2.88	1.64
11	288	2.40	1.20
12	217	3.63	2.45

PHINHOS and IKEMORI, 1980; AHUJA and LIBBY, 1993a and b). Adding a large number of intensively selected clones in the clone bank/trials, and determining the cluster behavior and genetic distance would help to maintain genetically diverse population of high yielding clones. Using the material from such clone gene bank, it is possible to develop a breeding programme with selected individuals of desired constitution. It is emphasized that clonal strategy should form part of overall genetic improvement programme. However, the inadequate progress made in genetic improvement need not *per se* limit clonal strategy if the selection of clones is carried out from

Table 2. – Cluster distance and genetic divergence relationship matrix for 12 clusters of *Casuarina equisetifolia.*

Cluster No.	1	2	3	4	5	6	7	8	9	10	11	12
1	**1.24**											
2	2.21 (M)	**0.00**										
3	4.26 (M)	6.33 (H)	**0.57**									
4	3.79 (M)	5.80 (H)	1.15 (C)	**0.54**								
5	4.18 (M)	6.20 (H)	1.31 (C)	1.05 (C)	**0.00**							
6	3.34 (M)	5.38 (H)	1.16 (C)	0.85 (C)	1.20 (C)	**0.61**						
7	2.96 (C)	4.93 (M)	1.78 (C)	1.05 (C)	1.57 (C)	0.92 (C)	**0.69**					
8	2.51 (C)	4.52 (M)	1.97 (C)	1.85 (C)	2.15 (C)	1.29 (C)	1.40 (C)	**0.74**				
9	2.50 (C)	4.21 (M)	2.82 (C)	2.12 (C)	2.93 (C)	2.05 (C)	1.51 (C)	2.03 (C)	**0.00**			
10	5.15 (H)	7.18 (H)	1.50 (C)	1.41 (C)	1.43 (C)	1.99 (C)	2.33 (C)	3.08 (M)	3.35 (M)	**0.62**		
11	6.41 (H)	8.45 (H)	2.55 (C)	2.71 (C)	2.55 (C)	3.22 (M)	3.57 (M)	4.31 (M)	4.52 (M)	1.38 (C)	**0.68**	
12	5.06 (H)	6.72 (H)	3.37 (M)	2.49 (C)	2.79 (C)	3.05 (M)	2.72 (M)	3.97 (M)	3.05 (M)	2.50 (C)	3.05 (M)	**0.00**

Figures in bold indicate intra-cluster distance.
Figures in parenthesis indicate divergence relationship H : Highly divergent; M : Moderately divergent and C : Closely divergent

diverse population so that the genetically broad based productive clones are maintained (GURUMURTHI *et al.,* 1994). The approach would help in channelising the productivity efforts of the population towards higher value without significantly affecting the genetic variation.

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Evaluation of Genetic Diversity in the Himalayan Poplar Using RAPD Markers

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Summary

The current investigation reports the evaluation and identification of genetic diversity in *P. ciliata* using the RAPD assay. Eighteen random decamer primers were used to assess variation within twenty five different clones, representing various provenances from the Himalayan region. A total of 159 amplification products were obtained of which 111 were polymorphic while the remaining were monomorphic in nature. Informative primers producing high multiplex ratio were identified from the study. The potential utility of these primers for large scale screening of germplasm and designing conservation strategies in the species has been discussed. The JACCARD's similarity coefficient and the UPGMA clustering method were employed to construct the phylogenetic tree. The dendrogram revealed a high level of variation between the clones which was found to lie in accordance to the diversity observed using morphological data. Two distinct clusters namely C1 and C2 were identified. The cluster C1 comprised of twenty three of the twenty five accessions and was thus designated to be the major cluster while C2 consisted of only two clones and was thus considered to be a minor cluster. The major cluster C1 was comprised of distinct sub-clusters which were found to be in concordance to their geographical distribution. Highest similarity within the major cluster was detected between the clones Katrain and Karain Bihal while the clone Lidder was found to be the most distinct. Bootstrap analysis and principal coordinate (PCO) analysis was performed which supported the pattern of clustering in the dendrogram. The clustering of the other clones in relation to their geographical location has been discussed.

Key words: Populus ciliata, Salicaceae, RAPD, conservation strategies, genetic diversity, intrapopulation variation.

Introduction

Poplars, members of the willow family, are known to comprise nearly 35 species, classified into five sections to which two more sections have been added (FAO, 1979; KHOSLA and KHURANA, 1982). Of the different species, *P. ciliata,* belonging to the section, Ciliata is one of the lesser known species being endemic to the temperate Himalayan belt of the Indian sub-

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