Population structure of *Dendrocalamus stocksii* along its Geographical distribution

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Abstract: *Dendrocalamus stocksii* is a commercial, domesticated and endemic bamboo species of Western Ghats, India. This non-seeding, vegetatively propagated species is geographically distributed between 12° to 17.5° North latitudes. A survey was conducted to understand the morphological variation for culm and clump characters along its geographical region. A total of 100 clumps (locations) were randomly evaluated in this region and these identified accessions were grouped into ten populations based on their latitude and longitude values. These ten populations varied for clump and culm characters with number of old culms positively influencing culm recruitment (r=0.509**). Among these demarcated populations, Sirsi population had comparatively better culm diameter and internode lengths followed by Ratnagiri and Sindhudurg populations. The clumps selected from Chandgad region were solid upto 7.06 m from the base while rest of the populations had solid clumps ranging between 3 to 5 m from the base. Height of solidness in culms was positively correlated with culm diameter to culm wall thickness ratio (0.391**) and latitude (0.319*), while, annual precipitation negatively influenced height of culm solidness (-0.246*). ISSR assisted diversity analysis augmented populations clustered according to their morphological and geographical variations.

Keywords: *Dendrocalamus stocksii*, Population, morphology, culm, clump, genetic variation

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Introduction

Dendrocalamus stocksii is an extremely manageable bamboo species with great economic and ecological importance (Singhal and Gangopadhyay, 1999) finding large-scale utilization in scaffolding, crafts, construction, basket making, umbrella handles and poles. Culms of this species are thornless with non-prominent nodes and better cw/cd (culm wall thickness to culm diameter) ratio, making it the most suitable species for furniture and construction industry in the lower diameter (< 2 inch) category. This species is endemic to Western Ghats of India (12° to 17.5° North latitudes) and is a widely domesticated species, usually incorporated in field bunds/farm boundaries and in homesteads (Viswanath *et al*., 2013).

The elevation gradients along the Western Ghats create variations in environmental and climatic conditions. Environment has long been recognized as playing a key role in both the determinants of phenotype in plants through effects on survival, growth, and reproduction and in the longer term, by acting as a major selection force affecting plant genotypes. Climate-related natural selection leads to local adaptation and differentiation of tree populations along climatic gradients (Howe *et al*., 2003). The prolonged influence of a climatic condition usually modifies and stabilizes a particular genetic character. Growth behaviour of *D. stocksii* which is exclusively distributed along the Central part of the Western Ghats of India may have stabilized at different locations.

Previous studies have indicated that *D. stocksii* has problems in seed setting coupled with sporadic

flowering behaviour. Dichogamy and protogyny is observed in *D. stocksii*, where the gynoecium matures 3 - 4 days before androecium preventing self-pollination (Beena *et al*., 2007). At the same time, considerable evidence is present on the role of out-crossing in determining patterns of genetic diversity and amounts of gene flow among populations (Hamrick and Godt, 1996). Studies have shown that the pattern of genetic variation among populations for growth potential follows closely the pattern of variation in the environment (Linhartand, 1996; Rweyongeza *et al*., 2007). Acquisition of sufficient information on the extent and pattern of genetic diversity, population differentiation occurring over geographical ranges, and understanding of the ecological and genetic relationship among individuals and populations are essential for establishing guidelines on conservation and utilization of the genetic resources of a species (Lee *et al*., 2000). Thus, tree populations typically show moderate to strong local adaptation despite high levels of gene flow (Howe *et al*., 2003; Savolainen *et al*., 2007).

The distribution of *D. stocksii* across a gradient of diverse ecological and climatic conditions may have caused natural variation in many of its morphological and genetic characteristics.

Materials and Methods

Study area

A survey was conducted to ascertain morphological variations in *D. stocksii* along the entire area of its distribution in the Western Ghats of India (12^{o} to 17.5^{o} North latitudes). *D. stocksii* clumps were randomly marked for evaluation, a distance of 10 km was maintained between two clumps for sampling. The sites selected were representative of their area of distribution and corresponded to different environmental conditions. A total of 100 clumps were evaluated covering a span of 1200 km from Ratnagiri, Maharashtra in North to Kasargod, Kerala in South. The sampled clumps were marked on map by recording their Latitude/ Longitude along with their altitude above mean sea level by using a GPS instrument. The rainfall data was collected for the respective blocks was obtained from the Meteorological Department. Soil texture information of the identified locations was retrieved from the Soil Resource Maps published by National Bureau Soil Survey and Land Use Planning, Nagpur (Anonymous, 1981).

Morphological Variation

Clump (a unit comprising of numerous culms) parameters viz. clump diameter, clump height, total number of standing culms (old and new), total number of harvested culms (single individual stem in a clump) and number of current year culms (new culms) were recorded. Clump diameter was evaluated by averaging the North-South and East-West clump diameters. Total number of standing culms and stumps (remains of the harvested culms) along with the current year emergents (culms with sheaths) were recorded. Five random culms each from matured and new emergent categories were evaluated for culm basal diameter, diameter and length of 5th internode using a digital vernier caliper.

One matured culm each from the identified clumps was extracted from the base and its total height and commercial height (height upto 20 mm culm diameter) was measured using a meter tape. The sampled culm was cross cut at various heights from the base and the height upto which the solidness was present was measured using a meter tape. At the cross cut portions, culm diameter and culm wall thickness was measured using a vernier caliper to derive the culm wall thickness to diameter ratio.

Genetic diversity

Genetic diversity was estimated among the populations by sampling DNA from tender leaves and by adopting the extraction procedure described by Doyle and Doyle (1987). The collected tender leaves were macerated and mixed with extraction buffer (Tris-HCL, EDTA based) and water bathed for 45 minutes at 65^{0}C. The pellet was collected after overnight incubation of supernatant after mixing with chilled Isopropanol. Later the pellet containing DNA were re-suspended in 50 µl of 1xTE buffer. A set of ISSR 15 primers composed wholly of defined, short tandem repeat sequences with anchor, and representing different microsatellites (di and tri-repeats) were used as generic primers in

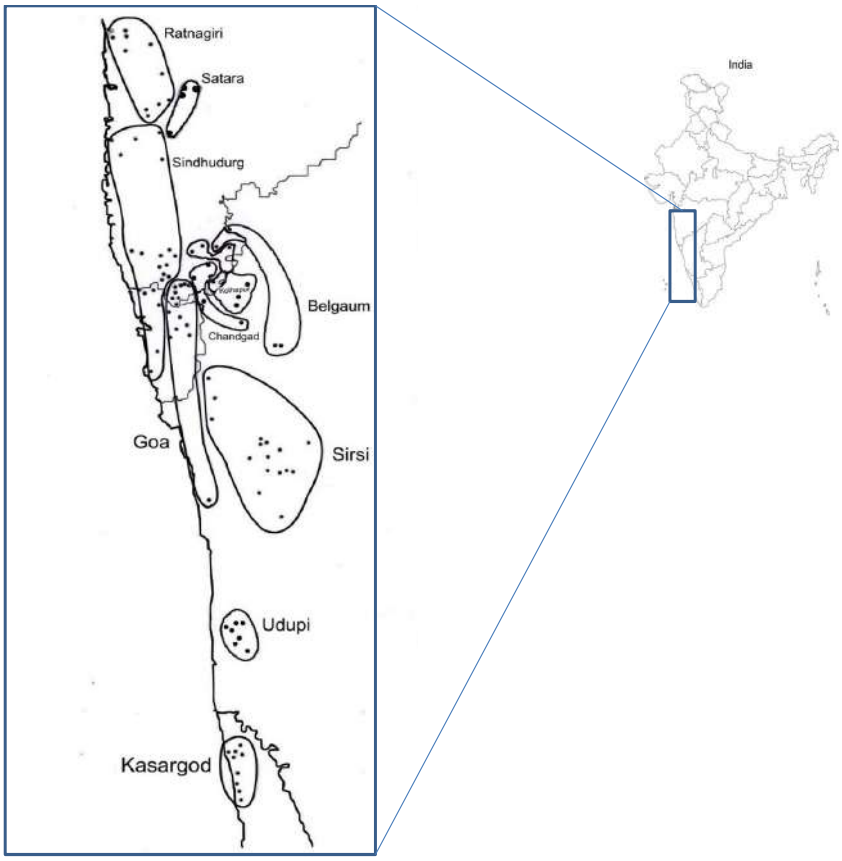

Fig 1. Clustered populations of *D. stocksii* along the Western Ghats

PCR amplification of inter simple sequence repeat regions (Adawy *et al*., 2002). The amplified DNA were separated on agarose gel electrophoresis by using a bromophenol blue tracking dye and compared with standard DNA ladder used was Φ x174/ Hae III digest which ranged from 310 bp-1353 bp.

Statistical analysis

Principal Component Analysis (PCA) was used to study variability of location and environmental factors based on latitude, longitude, elevation and mean annual rainfall. The populations were demarcated based on the influence of locations factors on the eigenvalue by performing a Ward's hierarchical cluster analysis for the location parameters of all the clumps and a dissimilarity matrix was computed and subjected to an agglomeration method using the average linkage clustering between groups. All the evaluated genotypes, differences among populations for various variables were calculated using analysis of variance (GLM procedures). Correlation between population means for each morphological trait and environmental factors such as altitude, latitude, longitude and precipitation were studied using Spearman's non-parametric correlation coefficient. This correlation coefficient is adequate for samples of small size and non-normal distributions. As the number of replications (clumps) in each population was unequal the DMRT (Duncans Multiple Range Test) was performed by estimating the critical range for each population based on their number of means. The data was analyzed using SAS 9.3 statistical software and the culm count data was log transformed before statistical analysis.

Results

Distribution of Populations: *D. stocksii* species was widely domesticated along its geographical region of Western Ghats and is maintained as isolated clumps on farm bunds, homesteads, farm boundaries and as block plantations. As this species does

Table 1. Populations formed as per the clusters of *D. stocksii* accessions formed for longitude and latitude factors

Sl. No.	Populations	Number of clumps sampled	Longitude (East)	Latitude (North)	Elevation (msl)	Annual Precipitation (mm)	Soil texture
1	Ratnagiri	10	73°22’	17°34’	126.71	3653.30	Sandy clayey loam with gravel
2	Satara	4	73°50’	17°05’	512.80	1797.84	Clayey soil
3	Sindhudurg	22	73°47’	15°80’	129.90	3320.97	Sandy clay loam with gravel
4	Goa	15	74°06’	15°29’	102.54	3347.00	Sandy clay loam with gravel
5	Kolhapur	8	74°21’	15°70’	744.60	2014.74	Clayey soil
6	Belgaum	4	73°34’	15°68’	637.28	1447.87	Loamy soil
7	Chandgad	5	74°18’	15°47’	853.70	1883.6	Clayey soil
8	Sirsi	15	74°68’	14°38’	584.66	2672.7	Gravelly clay
9	Udupi	7	74°53’	13°18’	88.42	4119	Clayey loam soil
10	Kasargod	10	74°98’	12°46’	146.49	1410.0	Clayey loam soil

not produce viable seeds, the existing germplasm has been extensively propagated through the traditional offset method (vegetative) and conserved by the local inhabitants of this region. All the 100 accessions collected in the survey were from farmers and forest dwellers located in various eco-geographical zones of the Western Ghats.

The 100 evaluated phenotypes of *D. stocksii* were grouped into populations, based on location attributes viz. longitude, latitude, precipitation and elevation. PCA (Principal Component Analysis) conducted for these location attributes revealed that longitude and latitude factors had eigenvalue > 1 and together constituted 91.12 percent of the total variation in geographical locations of *D. stocksii* phenotypes. All *D. stocksii* phenotypes were grouped into 10 populations by using latitude and longitude factors in thecluster analysis. The clustered groupings of *D. stocksii* revealed that these 10 populations were spread between 73°22’ to 74° 98’ E longitude and 12°46’ to 17°34’ N latitude (Fig. 1). These populations had variations in the eco-geographical conditions with respect to elevation, precipitation and soil type (Table 1). Annual precipitation varied between 1410 mm to 4119 mm among these populations along the Western Ghats at an elevation ranging between 88.42 to 853.7 m above mean sea level. The number of accessions in a population ranged between 4 - 15 and soil texture quality was sandy clay loam type in populations like Ratnagiri and Sindhudurg, while it was clayey type in Satara and Kolhapur.

Clump parameters: Culm production potential of clumps located in various populations varied significantly. Standing culms in a clump were strongly represented by old culms (70 percent) in all the populations. Culm growth potential of *D. stocksii* populations in Western Ghats varied among the populations. Clumps in Kasargod population had more number of old and current year culms while clumps in Belgaum along with that of Satara had less number of old and new culms (Fig. 2). The number of old culms positively influenced new culm production of the identified clumps along the Western Ghats (r=0.509**) while there existed no relation with other location parameters for culm production.

Culm parameters: Culm parameters varied significantly between the 10 populations of *D. stocksii*. Among various culm parameters, both old and new culms sampled from Sirsi population had comparatively better culm diameter and internode lengths followed by Ratnagiri and Sindhudurg populations (Table 2).

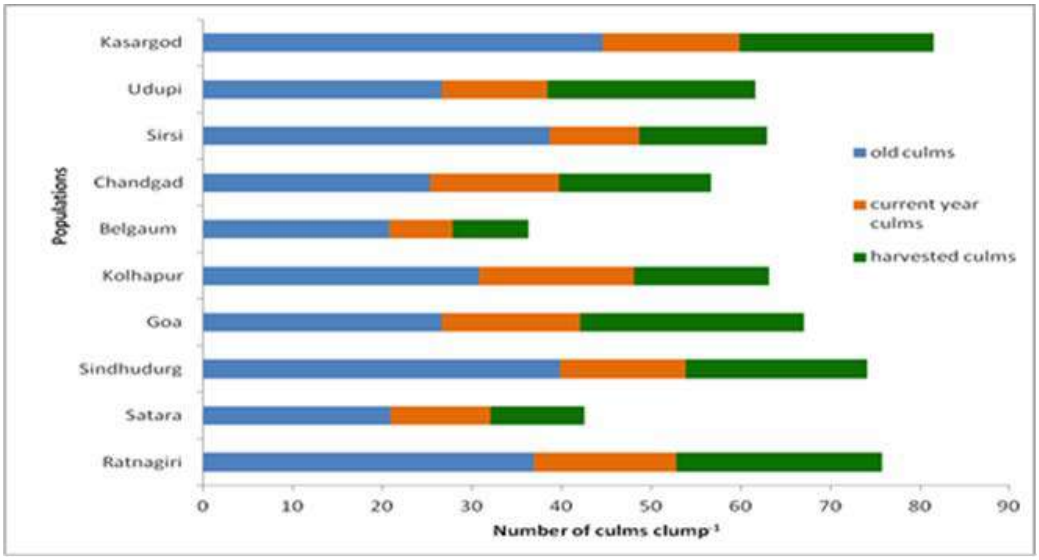

Fig 2. Standing culms and extraction frequency in the clumps of population of *D. stocksii* along the area of distribution

Basal diameter of old culms in five populations viz. Satara, Kolhapur, Belgaum, Udupi and Kasargod was less than 45 mm. Culm diameter to wall thickness ratio (cd/wt) of populations varied and among them culms present in the populations of Chandgad and Satara produced thick walled culms having a cd/wt of 0.54 and 0.51 respectively. Interesting feature was observed in the extent of culm hollowness in this species, clumps selected from Chandgad region were solid upto 7.06 m from base while rest of the populations had solid clumps ranging between 3 to 5 m from the base. Current year culms had 1 to 4 percent more basal diameter than old culms in most of the clumps, while in Sindhudurg, Goa, Chandgad and Sirsi populations the current year culms had lower diameters than old culms.

Total culm height ranged between 12.06 to 9.06 m and culms from Sirsi and Kasargod had more than 12 m height, while, culms in Chandgad and Satara had less than 9.5 m height. Commercial height (height up to 20 mm diameter) of this species ranged between 8.73 to 6.30 m and comprised of 70 percent of stick length. Sirsi population produced culms with better basal diameter, internode length and internode diameters (Fig 3&4), while, Chandgad populations produced culms with lower internode diameter and lengths.

Spearman Correlation Matrix revealed that the culm height was positively correlated with culm diameter and internode length parameters while it was negatively correlated with height of culm solidness (-0.217*) and culm diameter to wall thickness ratio (-0.344*). Height of solidness in culms was positively correlated with culm diameter to culm wall thickness ratio (0.391**) and latitude (0.319*), while, annual precipitation negatively influenced height of culm solidness (-0.246*). Basal diameter of the culms positively correlated with internode length and diameter among both old and new culms. Further, culm diameter of old culms negatively correlated with culm diameter to wall thickness ratios. Culm diameter to culm wall thickness ratio was negatively correlated with annual precipitation (-0.377*).

Genetic Diversity

Jaccard's Genetic Similarity Values: Jaccard's Similarity coefficient values between populations revealed that Belgaum and Kolhapur populations (0.40) had a high similarity value while a low similarity was observed between Sirsi and Chandgad (0.119) populations (Table 3). The similarity value of Kasargod population which was located in extreme South of the study area (Western Ghats) ranged between 0.167 (Chandgad) to 0.252 (Goa).

Table 2. Culm parameters of populations of *D. stocksii* along the Western Ghats

Populations	Basal diameter of new culms (mm)	Basal diameter of old culms (mm)	Fifth internode diameter of new culms (mm)	Fifth internode diameter of old culms (mm)	Fifth internode length of new culms (mm)	Fifth internode length of old culms (mm)	Culm wall thickness (mm)	Culm diameter to wall thickness ratio	Culm height (m)	Commercial height (m) (>=20 mm diameter)	Height of solidness
Ratnagiri	47.70^{ab}	45.88^{ab}	38.86^{ab}	37.70^{abc}	33.92^{c}	30.68^{c}	9.95^{c}	0.30^{d}	11.85^{ab}	8.27^{ab}	1.45^{b}
Satara	44.62^{c}	42.91^{bc}	35.93^{cd}	35.96^{bcd}	30.42^{d}	33.23^{bc}	15.25^{b}	0.51^{a}	9.54^{abc}	7.28^{ab}	5.28a^{b}
Sindhudurg	44.86^{bc}	45.96^{ab}	38.39^{ab}	38.07^{abc}	33.50^{c}	32.36^{bc}	9.98^{c}	0.32^{cd}	10.77^{abc}	7.74^{ab}	5.46^{ab}
Goa	44.56^{c}	46.20^{ab}	37.99^{abc}	38.88^{ab}	33.74^{c}	33.28^{bc}	9.26^{c}	0.30^{d}	10.41^{abc}	7.40^{ab}	3.04^{ab}
Kolhapur	42.72^{cd}	42.00^{cd}	35.13^{d}	34.66^{cd}	30.48^{d}	32.44^{bc}	11.20^{c}	0.39^{b}	9.15^{bc}	6.55^{ab}	5.15^{ab}
Belgaum	45.09^{bc}	43.72^{bc}	37.33^{bcd}	35.84^{bdc}	37.00^{b}	32.42^{bc}	14.17^{b}	0.38^{bc}	11.63^{abc}	7.18^{ab}	3.40^{ab}
Chandgad	43.34^{cd}	45.90^{ab}	35.30^{d}	36.39^{bcd}	28.07^{d}	30.52^{c}	18.27^{a}	0.54^{a}	9.06^{c}	6.30^{b}	7.06^{a}
Sirsi	48.10^{a}	48.79^{a}	39.88^{a}	40.10^{a}	40.74^{a}	40.67^{a}	12.41^{bc}	0.33^{bcd}	12.28^{a}	8.73^{a}	2.47^{b}
Udupi	40.52^{d}	38.91^{d}	35.26^{d}	34.07^{d}	33.63^{c}	31.84^{bc}	9.42^{c}	0.27^{d}	10.91^{abc}	8.06^{ab}	0.82^{b}
Kasargod	44.40^{c}	40.58^{cd}	38.70^{ab}	35.49^{bdc}	34.29^{c}	33.71^{b}	9.99^{c}	0.27^{d}	12.06^{a}	8.25^{ab}	0.58^{b}

Table 3. Jaccard's Similarity coefficients between ten populations of *D. stocksii* in Central Western Ghats

	Chand-gad	Sa-tara	Sind-hudurg	Goa	Kolha-pur	Bel-gaum	Ratnag-iri	Sirsi	Udu-pi	Kasar-god
Chandgad	1.000									
Satara	0.150	1.000								
Sindhudurg	0.160	0.203	1.000							
Goa	0.184	0.230	0.198	1.000						
Kolhapur	0.212	0.225	0.167	0.200	1.000					
Belgaum	0.231	0.194	0.223	0.198	0.404	1.000				
Ratnagiri	0.183	0.157	0.243	0.226	0.179	0.178	1.000			
Sirsi	0.119	0.183	0.143	0.231	0.156	0.174	0.210	1.000		
Udupi	0.164	0.168	0.139	0.162	0.190	0.160	0.204	0.209	1.000	
Kasargod	0.167	0.212	0.171	0.252	0.193	0.243	0.219	0.213	0.229	1.000

UPGMA clustering between populations: The 10 populations of *D. stocksii* clustered in two main groups and first group comprised of Belgaum, Chandgad and Kolhapur (Fig. 4). In the second sub cluster, Ratnagiri and Sindhudurg were closely associated, while Sirsi, Goa and Kasargod were clubbed together. Moreover, in the same sub cluster, Udupi and Satara populations were isolated from the rest. The cluster pattern may be related with the variation in height of solidness observed between populations, wherein culms from Chandgad, Belgaum and Satara had better height of solidness. So also, Sirsi population contained culms with overall superiority in culm basal diameter and diameter and length at 5th internode.

Discussion

Distribution of populations: *D. stocksii* constituted an important component among farm and homestead boundaries along the Western Ghats implying its mass acceptance and adaptability along various elevation and precipitation gradients in the Western Ghats. Though the natural distribution of this species is in humid tropics with lateritic soil type, this species has a wide adaptability and comes up well in sub humid and semi-arid conditions under black and red soils as well (Rane *et al*., 2014). Earlier studies indicated that it is the most preferred species after *Bambusa bambos* and *Dendrocalamus strictus* by the farmers in Peninsular India (Anonymous, 1981).Its non-thorny nature and loosely spaced culms facilitates easy management so also it is widely recommended for cultivation and research by various national agencies like National Bamboo Mission by Government of India (Haridasan and Tiwari, 2008) . A vast distribution pattern of this species into various eco-geographical zones has resulted in the diversity for various characters.

The Principal Component Analysis resulted in the clustering of all 100 locations into ten populations along the Western Ghats on the basis of latitude and longitude. As the latitude and longitude govern climatic and growth behaviour of a species (Vajda and Venalainen, 2003), such type of clustering among the populations is possible. Meng *et al*., (2017) have concluded that latitude predicted trait variations throughout the distribution of *Euptelea pleiospermumin* China. Various environmental attributes like rainfall, types of vegetation and geomorphology of the region discriminate species into populations (Bottini *et al*.,2000). A similar observation in Pine revealed that eco-geological attributes like aridity indices, longitude,

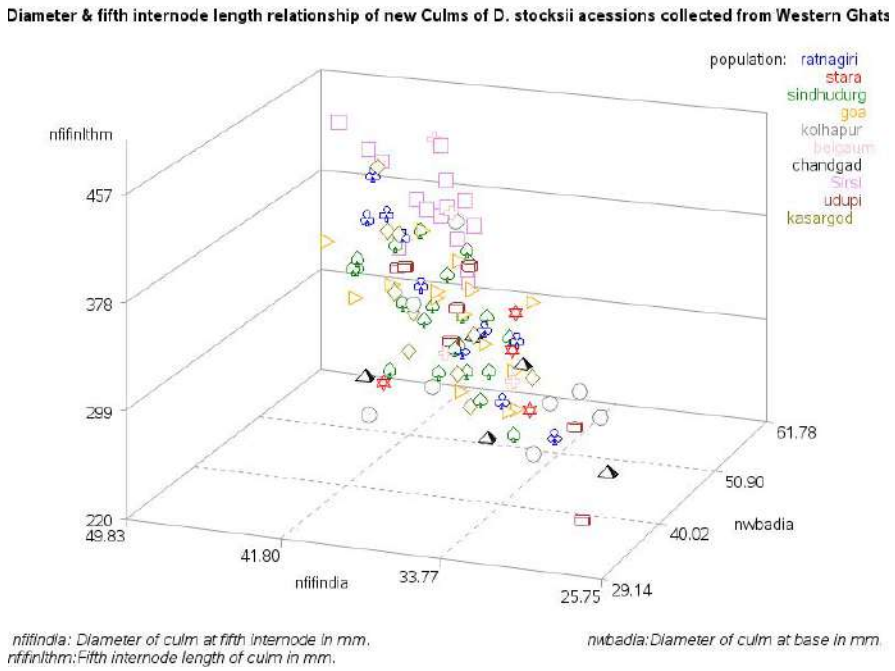

Fig 3. Diameter and internode lengths of new culms of *D. stocksii* accessions along the Western Ghats

and latitude explained most of the variance in PC1 and 39 percent of the variance in PC2 (Eckert *et al*., 2010).

Clump Characteristics: Culm production capacities of clumps varied among the populations and most of them were stocked with culms of more than one year of age. This is indicative that most of the clumps were subjected to selective harvesting in the region with a longer felling cycle was practiced. As this species is retained and cultivated by farmers owing to its multiple utility potential, a regular harvesting regime may have been conducted in these clumps. Belgaum and Satara populations had comparatively less culm density as these two elevated areas receive less precipitation as compared to other locations as they are present on the leeward side of the Western Ghats. It is well acknowledged that Western Ghats receive enhanced rainfall activity substantially under favorable conditions, with increasing trend on the windward and decreasing pattern on the leeward side. Due to such variation in rainfall pattern, homogeneity in rainfall distribution cannot be expected over the Western Ghats of India (Venkatesh and Jose, 2007). Though the present study did not show any significant correlation between rainfall and culm density, it is usually observed that precipitation affects distribution and limits growth of bamboo more than any other component of climate (Biswas, 1988). Further, culm recruitment among the clumps was influenced by number of old culms and is indicative that culm density mediates culm recruitment (Taylor and Zisheng, 1993).

Culm Characteristics: Clumps present in the populations of Sirsi, Ratnagiri and Sindhudurg produced culms with higher diameter and height. Among these populations, bamboos are cultivated in association with trees (Shastri *et al*., 2002). Similarly, variations in individual bamboo shoot biomass among various locations of *Fargesia yunnanensis* were associated with companion tree species and soil nutrient status (Wang *et al*., 2009). An exception was observed in culm diameters of Kasargod and Udupi populations, wherein, even with better precipitation, clumps produced comparatively smaller diameter culms and this may be due to soil type observed in the locations. The soil type in Udupi comprised of gravelly clay soil with surface compaction on undulating uplands, while the soil of Kasargod is of a well-drained clayey type (Anonymous, 1981). Soil physical factors such as slope of land, texture, bulk density, moisture holding capacity, temperature are the nonchemical properties of soil which influence bamboo produc-

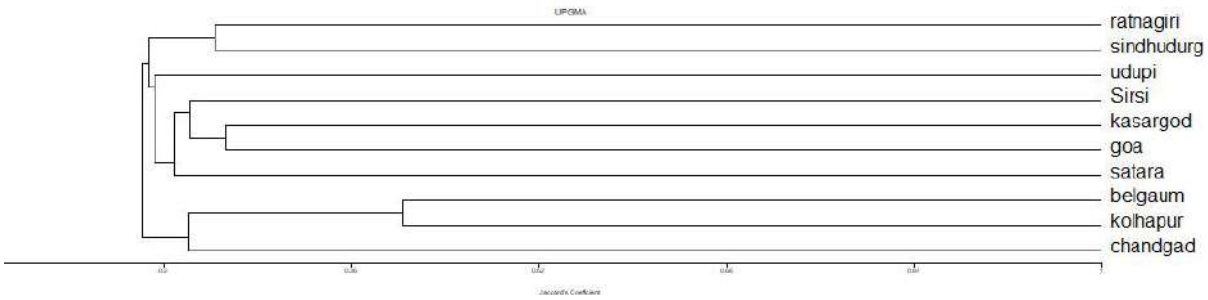

Fig 4. UPGMA dendrogram between ten *D. stocksii* populations

tivity(Kleinhenz and Midmore, 2001), moreover, high bulk density of clay soils negatively affects bamboo growth (Kleinhenz *et al*., 2003).

D. stocksii in most of the locations was present in homegardens comprising of evergreen tree species especially in Sirsi and Sindhudurg populations. These two locations have tree diversity values in comparable with tropical rain forest especially the home gardens (Shastri *et al*., 2002). So also, taller and thicker culms were observed under mixed evergreen and deciduous canopy than under homogenous canopies (Donald *et al*., 1991).

This species does not produce seeds (Seethalakshmi and Mukteshkumar, 1998) because of quick drying of anthers and lack of dehiscence and pollen deposition on stigmatic hairs. However, non-seeding behavior of a bamboo species does not have any influence on intraspecific diversity of a species, as was observed in Bambusa vulgaris (Ofori *et al*., 2006), which showed a clear pattern of variation among the individuals of evergreen and semi-deciduous populations. This species is exclusively present on farmers fields (Viswanath *et al*., 2016) in the form of cloned improved material as the farmers practice offset propagation after selecting a superior clump.

Basal diameter and internode length of this species was correlated positively and is a usual trend observed in trees. Latitude influenced height of culm solidness and fifth internode lengths of culms. Latitude negatively/positively influences a specific character of a species (Boulli *et al*., 2001). It was observed that, height of culm solidness was negatively correlated with annual precipitation and hollowness was nearly absent in the populations like Chandgad and Satara that received less rainfall. Earlier reports on factors influencing culm solidness are not available; however, solid stemmed province germplasm of wheat was collected from lower altitude comprising of higher temperature and shorter growing season in Turkey (Damania *et al*., 1997). This indicates that lower annual precipitation may have some influence on culm solidness.

Conclusion

D. stocksii is distributed along the Western Ghats on the farmers fields show variations with respect to its morphology and growth. This distribution pattern can be grouped into 10 populations that have marked geographical variations. Among the various population, Sirsi, Sindhudurg and Ratnagiri can be subjected to further evaluation to ascertain superior germplasm for further improvement. As these individuals are already selected by farmers and cloned, it may be easier to undertake improvement because there may be possibility of homogeneity and stability of a character within the population, which requires further analysis.

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