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MORPHOMETRIC DIVERSITY OF *PONGAMIA PINNATA* (L.) PIERRE CANDIDATE PLUS TREES A POTENTIAL AGROFORESTRY TREE

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ABSTRACT
Pongamia pinnata is an important biofuel-yielding tree species. It is identified as a sustainable substitute for petroleum fuel. A study was conducted to assess the distribution and extent of morphometric diversity among the population across major pongamia growing agro-ecological zones of Karnataka, Andhra Pradesh and Tamil Nadu. During the course of study, 232 accessions were selected for recording their morphometric traits. Significant differences ($P<0.05$) existed among all the zones studied for oil content. DIVA-GIS approach showed that provenances of Davanagere, Chitradurga, Kolar, Tumkur, Bengaluru Rural, Hassan and Chamarajnagar supported promising genotypes with high oil content. Diverse genotypes with respect to yield were recorded from provenances of Chitradurga, Kolar, Hassan and parts of Tumkur. Based on canonical correspondence analysis, altitude and soil directly correlated with the fruits per bunch and average seed yield. Based on the Euclidean distance and UPGMA method for qualitative morphological traits, candidate plus trees were grouped into two main clusters which were further subdivided into 4 sub-groups. Random grouping of accessions from different agro-ecological zones were observed. The present investigation suggested that the success of biofuel program in Karnataka could invariably be dependent on the selection of elite planting material across the highly populated regions of central and eastern dry zones of Karnataka as compared to the other agro-ecological zones.

KEY WORDS: Candidate plus trees, DIVA-GIS, morphometric diversity, zonal variation.

INTRODUCTION
An increase in demand for petroleum products in industrial and domestic sectors has caused serious concern about its future availability and supply for further use. Asian countries among the developing and under developing countries are likely to face the energy crisis following an increase in population and improvement in livelihoods (Wani *et al.,* 2006). The future energy needs of India could only be met by the supply of an alternative source of energy, the biofuel. In India which is identified as one of the biodiversity-rich countries, a large number of non-edible oil yielding species could be identified as the source of biofuel (Chhetri *et al.,* 2008; Durrett *et al.,* 2008). However, the productivity of these alternative sources of energy is limited. There is a need to oversee the availability of feedstock of biofuel species for the continuous production of biofuel (Ramesh and Gowda, 2009). Cultivation of the energy plantations particularly in wastelands could also serve as a micro-enterprise for poor Indian farming community (Kesari and Rangan, 2010; Savita *et al.,* 2010). At the same time, the wasteland that is generally unsuitable for cultivation of agriculture crops and hence could be utilized for the establishment of biofuel plantation. Amongst many tree species identified as the promising substitute for fossil fuel (Augustus and Seiler, 2001) *Pongamia pinnata* (Fabaceae) commonly known as honge or karanj is distributed widely in different agro-ecological zones of India. It is a medium-sized tree indigenous to Indian sub-continent and South East Asia and is also grown in Australia, the United States of America and China (Anonymous, 1969). It is tolerant to water logging, saline soils and can withstand harsh climatic conditions. Besides its domestic use, the plant species also has a number of applications in the field of medicine (Elanchezhiyan *et al.,* 1993; Shivanna and Rajakumar 2010), insecticide (Pavela, 2009) and animal husbandry (Prabhu *et al.,* 2002; Panda *et al.,* 2006). Karanjin extracted from seed provides value addition to pongamia oil (Vismaya *et al.,* 2010). Previous investigations have shown that pongamia oil could be used as an efficient substitute for petroleum fuel in diesel engine (Karmee and Chadha 2005; Venkateswara *et al.,* 2008). *Pongamia* starts bearing fruits at the age of 6-7 year with the seed yield of 9 to 90 kg per tree and seeds could be harvested after 42 weeks after flowering with maximum oil content (36 to 39 %) and other seed reserve materials (Pavithra *et al.*, 2012; Pavithra *et al.,* 2013; Pavithra *et al.,* 2014) The cultivation of this species offers a lucrative approach. The species is known to improve soil fertility (Scott *et al.,* 2008), prevent soil erosion, increase tree growth, foliar nitrogen and phosphorus status of neighboring trees (Singh, 2006). Presently, there is a great demand for pongamia seed and its oil with specific fatty acid content. However, the availability of seed oil is so less that it is not possible to cater to the needs of a variety of consumers. The enormous naturally available seed feedstock in southern districts of Karnataka have contributed to the lateral movement of seed oil to

numerous biofuel-based industries established in the neighboring states like Andhra Pradesh and Tamil Nadu.

Literature on the systematic documentation of the available elite genotypes of pongamia in Karnataka is very scanty. As part of the key initiatives to biofuel program in Karnataka, 'Biofuel Park' as a mega model was established in Hassan (Gowda *et al.,* 2009) to carry out systematic studies on nursery, production of biodiesel and marketing system, in addition to the identification of the elite genotypes for establishing the clonal orchards in different growing regions of Karnataka. In this context an attempt was made to conduct a survey of the availability of pongamia germplasm growing in the peninsular India consisting of transition zones of Karnataka, Andhra Pradesh and Tamil Nadu and identify the diversity of trees and elite trees among them capable of high seed and oil yield based on their morphometric traits. An attempt was made to predict the diversity and distribution of pongamia trees by DIVA- GIS application (Babu *et al.,* 2010; Hijmans *et al.,* 2001). The effect of environmental variables on the morphometric characteristics of pongamia germplasm was also determined.

MATERIALS & METHODS

Selection of study area

The choice of study area in which the candidate plus trees (CPT) have been selected was governed by the occurrence of vast pongamia population. The regions involving the boundaries of three southern Indian states in the peninsular India such as Karnataka, Tamil Nadu and Andhra Pradesh were selected for the identification of pongamia germplasm. Region-wise specific survey was conducted for the collection of seed samples during the harvesting period (Feb-March 2008 to 2010). Five agro-ecological zones were identified based on the availability of natural population of pongamia and their climatic conditions (arid and semi arid conditions) as the study area within these regions. The provenances assembled under each of the study area are detailed (Table1). Data on the temperature and rainfall relating to the study area were collected from department of Agro-meteorology, Gandhi Krishi Vignana Kendra, University of Agricultural Science, Bengaluru. The soil color was measured using the Munsell color book (Anonymous, 1994). The geographical location of each study area was documented using the Global Positioning System (GPS, Garmin eTrex Vista HCX, Taiwan).

Zone 1- Central dry zone of Karnataka state

The germplasm was collected from Davanagere (N14°10'16.7" - N14°44'25.8" to E75°40'20.04"- E76°24'25.8"; altitude of 543 - 698 m) and Chitradurga (N13°39'33.0"- N14°37'53.5" to E76°07'55.0" - E76°44¹ 12.3"; altitude of 510 - 765 m) provenances which has red soil and experienced the temperature of 38 - 40 °C and rainfall of < 400 mm. From this area, a total of 50 CPT were identified and marked.

Zone 2 - Eastern dry zone of Karnataka state

This zone included Tumkur, Bengaluru rural and Kolar provenances. The germplasm was collected from Tumkur (N13°03'00.8" - N13°50'05.4" to E76°34'14.0" - E77°08'42.5"; altitude of 670 - 860 m), Bengaluru rural (N12°27'17.9" - N12°44'29.0" to E77°12'56.8" - E77°31'36.9"; altitude of 697 - 900 m) and Kolar provenances (N13°00'36.0" - N13°49'02" to E77°31'01.7" - E78°12'8.08"; altitude of 734 - 970 m). These regions have mainly red soil, with occasional black and red rocky soil. The temperature ranged from 35 - 38 °C and the rainfall was <400 mm in Kolar, while it was 400 - 1000 mm in Tumkur and Bengaluru rural provenances. Totally, 84 CPT were selected from this zone.

Zone 3- Southern dry and transition zone of Karnataka state

The details of the provenances covered under this zone are: Hassan (N12°46'46.0" - N13°18'21.6" to E75°53'57.5" - E76°25'14.4"; altitude of 820 - 1100 m), Mandya (N12°29'42.9" - N12°34'9.5" to E76°50'04.5"- E77°02'55.5"; altitude of 629 - 709 m) and Chamarajanagar (N11°50'54.4" - N12°10'25.9" to E76°48'37.8" - E77°16'37.1"; altitude of 652 - 835 m). The soil type in this zone is mainly red soil and occasionally black soil in some regions. The temperature ranged from 27 - 30 °C and the annual rainfall was 400 - 1000 mm. Fifty seven CPT were selected from this zone.

Zone 4 - Transition zone between Karnataka and Andhra Pradesh

This zone included Anantapur and Chittor provenances (N13° 32'33.9" - N14°11'52.4" to E77°33'00.3" - E78°43'41.3"; altitude of 451- 907 m) which have trees on red soil with average temperature of 40 °C. These regions received a rainfall of < 400 mm. In this zone, 12 representative CPT were marked randomly.

Zone 5 - Transition zone between Karnataka and Tamil Nadu

Dharmapuri, Erode and Salem provenances (N11° 17'20.6" - N12°39'30.06" to E77°35'49.9" - E78°15'34.5"; altitude of 198 - 667 m) were identified in this zone. Trees were growing mainly on red soil with few on black soil. The temperature ranged from 30 - 34 °C and the rainfall from < 400 to 1000 mm. Twenty nine CPT was selected from these provenances.

Selection of candidate plus trees

The selection of pongamia trees was made by using single tree selection method based on the phenotypic assessment of agro-morphologically important traits (Anonymous, 1995). The trees were marked in different habitats like wasteland, bund, road-side, canal-side, farm-bund and backyard prevailing in the study area. Trees with desired characters were recorded as candidate plus trees. The phenotypic traits of marked trees were compared with those of trees growing nearer. Each CPT (aged above 7 years) was separated from the other by atleast 1-5 km. The seed yield of the CPT was determined with the help of local farmers and the CPT that were marked was subjected to the evaluation of certain morphometric traits like tree height, girth at breast height (GBH), number of main branches and sub-branches, canopy diameter and shape, branching pattern, fruit number per bunch and average seed yield per tree (Anonymous, 2007). Pods were collected uniformly from all branches of the tree. The pod characteristics like size, texture, thickness and tip were also recorded (Fig.1). The pod size was categorized into small (3 - 3.5 cm), medium (3.5 - 5 cm) and large (5 - 7.5 cm), pod texture into smooth and rough, pod thickness into bulged (0.1 - 1.5 cm) and flat (0.5 - 1 cm), pod tip into beaked and mucronate. Standard measuring tapes were used and pod size was determined using a Vernier caliper. The oil content of seeds collected from CPT were analyzed by soxtherm apparatus (Gerhardt, Germany) on dry weight basis. The triplicate seed (dry) samples were taken and extracted using petroleum ether (Merck, India; 40 - 60°C).

TABLE 1: *Pongamia pinnata* candidate plus trees (CPT) identified in different agro-ecological zones of the study area
[a] Data in parenthesis indicate the number of CPT marked in the respective area

Sl no.	Regions of collection / Provenances				
	Central dry zone of Karnataka (Zone 1)	Eastern dry zone of Karnataka (Zone 2)	Southern dry and transition zone of Karnataka (Zone 3)	Transtion zone between Karnataka and Andhra Pradesh (Zone 4)	Transtion zone between Karnataka and Tamil Nadu (Zone 5)
	Davanagere (24)[a] Chitradurga (26)	Tumkur (26) Bangalore Rural (17) Kolar (41)	Hassan (40) Mandya (7) Chamarajnagar (10)	Anantapur (9) Chittor (3)	Dharmapuri (15) Erode (3) Salem (11)
1	Jagalur (4)	Tumkur (4)	Hassan (8)	Anantapur (3)	Hosur (5)
2	Harappanahalli (4)	Tiptur (2)	Arakalagudu (4)	Penukonda (3)	Krishnagiri (6)
3	Harihara (3)	Gubbi (8)	Arsikere (11)	Kadiri (3)	Dharmapuri (4)
4	Honnali (3)	Kunigal (2)	Channarayapatna (7)	Madanapalli (2)	Erode (3)
5	Chennagiri (4)	Sira (7)	Belur (6)	Chittor (1)	Salem (11)
6	Davanagere (6)	Chikkanayakanahalli (3)	Holenarsipura (4)		
7	Molakalmuru (4)	Kanakpura (8)	Mandya (3)		
8	Challakere (7)	Channapatna (8)	Maddur (4)		
9	Chitradurga (4)	Ramnagara (1)	Kollegala (4)		
10	Hosadurga (5)	Chikkaballapur (8)	Yallandur (3)		
11	Holalkere (3)	Sidlaghatta (7)	Chamarajanagar (2)		
12	Hiriyur (3)	Kolar (3)	Gundlupet (1)		
13		Bangarpet (3)			
14		Srinivaspura (4)			
15		Chintamani (2)			
16		Bagepalli (5)			
17		Gudibande (5)			
18		Gauribdnoor (4)			

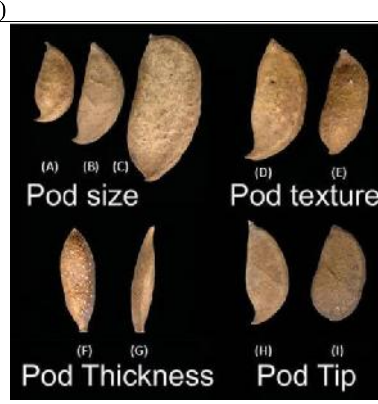

FIGURE 1. Pod characteristics in candidate plus trees of *Pongamia pinnata*
Pod size: (A) Small (3 - 3.5 cm) (B) Medium (3.5 - 5cm) (C) Large (5 - 7.5 cm)
Pod texture: (D) Smooth (E) Rough
Pod thickness: (F) Bulged (0.1- 1.5 cm) (G) Flat (0.5 - 1cm)
Pod tip: (H) Beaked (I) Mucronate

Statistical analysis of data

The mean performances of the CPT for morphometric traits were studied. Duncan Multiple Range Test (DMRT) was used to compute the data and for comparison of the means of selected morphometric traits (MSTAT-c, Ver. 5.1). The area consisting of CPT was mapped using DIVA-GIS software (Ver. 5.2). The average seed yield and oil content performance of CPT in diverse area were mapped by Shannon diversity index using DIVA-GIS. The point to grid map was generated by the simple neighborhood method. Colors of the grid are indicative of the extent of diversity in the germplasm collected across different agro-ecological zones. Canonical correspondence analysis (CCA) (Ter Braak, 1986) with biplot, eigenvalues, total variance, correlation values, ordination scores for all the CCA axis were worked out for the morphometric traits and environmental variables like soil and altitude (PC-ORD, Ver. 5) (McCune and Mefford, 1999). Based on the qualitative morphometric traits, Euclidean distances were calculated and a dendrogram was constructed using UPGMA (Unweighted Pair Group Method Using Arithmetic Averages) (Sneath and Sokal, 1973) using STATISTICA (Ver. 6).

RESULTS & DISCUSSION

Distribution and morphometric diversity of Pongamia pinnata across different agro-ecological zones

In the present study, vast populations of pongamia have been documented in the arid, semi-arid and transition zones of Karnataka, Andhra Pradesh and Tamil Nadu though very few trees were also recorded in the coastal and hilly zone of southern Karnataka with low seed yield and oil content (data not shown). These CPT were selected for studying the pod and seed traits from different provenances from arid, semi-arid and transition zones with varying habitat. Two hundred and thirty two accessions of pongamia CPT were collected from different agro-ecological zones of Karnataka, transition zones of Karnataka, Andhra Pradesh and Tamil Nadu. The accessions were grouped based on the morphometric and yield traits (Table 2). Most (85 %) of the marked trees from different agro-ecological zones occurred on red soil and a few CPT from eastern dry and southern dry zones found on black and red rocky soil. The trees found abundantly along the bunds of farms demarcate their boundary, on back-yard of houses and avenues. Twenty-nine per cent of accessions were in the age group of 10-20 year followed by 23 % of CPT in the age group of 20 - 30 year and a few accessions (7 %) above 50 year. The tree height of 6- 9 m was recorded in 38 % of CPT and 9 - 12 m in 26 % of the CPT. The GBH was high in zone 2 with more than 100 cm recorded in 55 % of accessions. Most of the CPT had less than 3 main branches (90 %). The maximum numbers (53 %) of CPT from zone 2 had 5-10 sub-branches in comparison with those in other zones. Canopy diameter of 7-15 m was recorded in 49 % of CPT with most of them having circular canopy and a few (3 %) had more than 15 m canopy diameter. Maximum number of CPT from zone 2 recorded high canopy diameter. Seventy-seven per cent of CPT showed drooping branches. Number of fruits per bunch was < 10 (53 %) followed by 10 -15 fruits per bunch (34 %) and few CPT (12 %) with more than 15 fruits per bunch. Zone 2 had maximum number of CPT yielding 10 - 15 fruits per bunch. Most of the CPT from zones 4 and 5 produced less than 15 fruits per bunch. Fifty-one per cent of the CPT produced rough pod with mucronate tip. Most of the CPT from all the zones yielded medium-sized pods (62 %) followed by small pods (31 %) and a few large pods (6 %). Most of the CPT (51 %) produced pods with good seed filling. Most of the CPT (80 %) yielded one seed per pod and few CPT (20 %) with both one and two-seeded pods. The pods with two seeds had one bigger seed at the stock than the other at the tip of the pod. There was no expectable relationship between pod characters (size, texture, thickness and tip) and number of seeds per pod. Based on the seed yield, the CPT was categorized into three groups- 20-40 kg (31 %), 40-60 kg (34 %) and > 60 kg (35 %). The CPT from zone 2 as compared to other zones yielded more than 60 kg of seed. The oil content of single and two seeded pods showed no variation within the CPT. The majority of the CPT produced oil content between 30-35 % (62 %) and one accession each from zones 2 (CPT 97) and 3 (CPT 4) produced more than 40 % oil. In tree species, the identification of superior genotypes among the germplasm collection is dependent on observations over a period of time. Most morphometric traits are considered important in predicting their contribution to the final yield of the plant along with their heritability (Ratree, 2004). The diversity of pongamia trees varied depending on different morphometric traits. The CPT of pongamia performed better in red soil than in the other soil types. The age of the CPT did not correlate with the oil yield. These observations are on par with the results reported by Munoz-Valenzuela (2007) in *Azadirachta indica* collected from Yaqui valley in Mexico. Most of the pongamia accessions had a medium plant height of 6-9 m with drooping branches such of them facilitated an easy and efficient harvesting of pods. Trees with medium height and many branches could be an important desirable characteristic of the oilseed yielding trees (Babu *et al.,* 2007). Similar observations were reported during the selection of *Jatropha curcas* genotypes for breeding program by Mishra (2009). Since pongamia is mainly used for seed yield and oil content, selection based on height need not be an important criterion. The canopy diameter of 7- 15 m could provide sufficient space for spread of 5 to 10 sub-branches with high fruit bearing ability. The number of fruits per bunch less than10 appears to balance the number of sub-branches (5-10) and correlated with oil content (30-35 %). In *Jatropha curcas* also the study of specific plant traits is important in predicting the promising accessions for the purpose of germplasm conservation and maintenance (Sunil *et al.,* 2008). In the present study, based on the seed yield superior pongamia genotypes were selected from the respective zone - CPT 138 (zone 1), CPT 92 (zone 2), CPT 12 (zone 3), CPT 229 (zone 4) and CPT 208 (zone 5). The present study indicated that the availability of natural genetic variation within pongamia population could be exploited for the tree improvement program. Bedell (2006) opined that in any tree improvement program, the first step begins with the defining of traits that needs improvement. Selection of pongamia for breeding involves the identification of genotypes superior in seed and oil yielding abilities, testing of off-spring in progeny trials, seed production ability of off-spring and production of biofuel from high oil yielding seeds. A maximum number of CPT from zone 2 (eastern dry zone of Karnataka) were identified for their superior phenotypic characters like GBH, drooping branches, 5-10 sub-branches, large canopy diameter, 10-15 fruits per bunch and high seed yield with 33-38 % oil content. Hence, Tumkur, Bengaluru rural and Kolar provenances of zone 2 could be identified as the good locations for the selection of superior genotypes, based on the morphometric traits.

Mean performance of CPT for morphometric traits

Analysis of mean performance of the candidate plus trees for morphometric traits indicated a significant zonal variation with respect to height, girth of the tree, number of main branches, number of sub-branches, fruits per bunch, average seed yield and oil content (Table 3). Variation among different zones of Karnataka, Andhra Pradesh and Tamil Nadu was clearly evident ($P < 0.05$). Zone 2 showed high mean value for tree height (12.44 m), while zone 4 showed lowest mean value (7.62 m). The CPT from zone 5 had maximum GBH (170.7 cm) while those in zone 4 showed the minimum (65.84 cm). Zone 1 had CPT with highest number of main branches (4.00) and sub-branches (8.84) as compared to those of other zones. Maximum fruits per bunch (12) were produced in CPT from zone 2 and minimum (7) was observed from zone 5. Mohapatra and Panda (2010) reported a similar kind of variation with respect to the morphology in *Jatropha curcas* collected from Bhubaneswar. The CPT from zone 2 produced high seed yield (61.67 kg) and that from zone 4 low (38.34 kg). Zone 2 was also associated with high mean value (34.86 %) for oil content, while zone 5 with low (32.20 %) oil content. Similar study based on yield performance of natural populations of almond trees from Morocco was reported by Ait Aabd *et al.*, (2011). Kaura *et al.*, (1998) reported variation in morphology and oil content of *Azadirachta indica* seeds from different agro-ecological zones. Significant zonal variations in morphometric traits in tree species could be attributed to annual rainfall pattern, temperature and soil type. Zones 1 and 2 showed highest mean value for various morphometric traits. Similar variations in agro-morphological traits of shea tree *Vitellaria paradoxa* from four agro-ecological zones of Cameroon was reported by Diarrassouba *et al.*, (2007).

Diversity assessment using DIVA-GIS

DIVA-GIS was used to generate maps of the distribution and diversity of the CPT (Fig. 2) and further mapping was done based on point collections using the spatial data of average seed yield and oil content. The high diversity index was observed in collections (Fig. 3A) from parts of central and eastern dry zone of Karnataka followed by southern dry transition zone of Karnataka. A very low diversity index was obtained in transition zones between Karnataka and Andhra Pradesh, Karnataka and Tamil Nadu. This further indicated that the diversity of pongamia was high in the drier zones located in Karnataka.

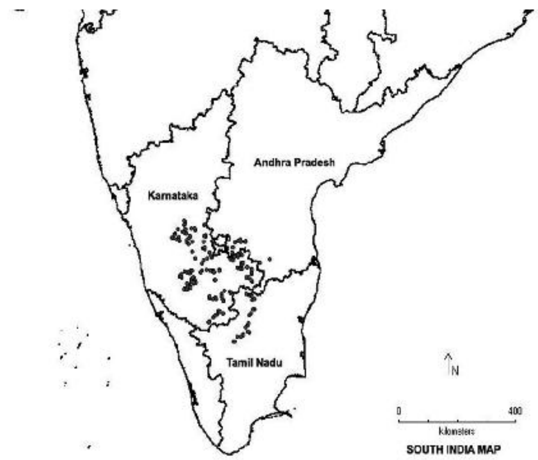

FIGURE 2. Location mapping of *Pongamia pinnata* CPT marked in different agro-ecological zones of the study region

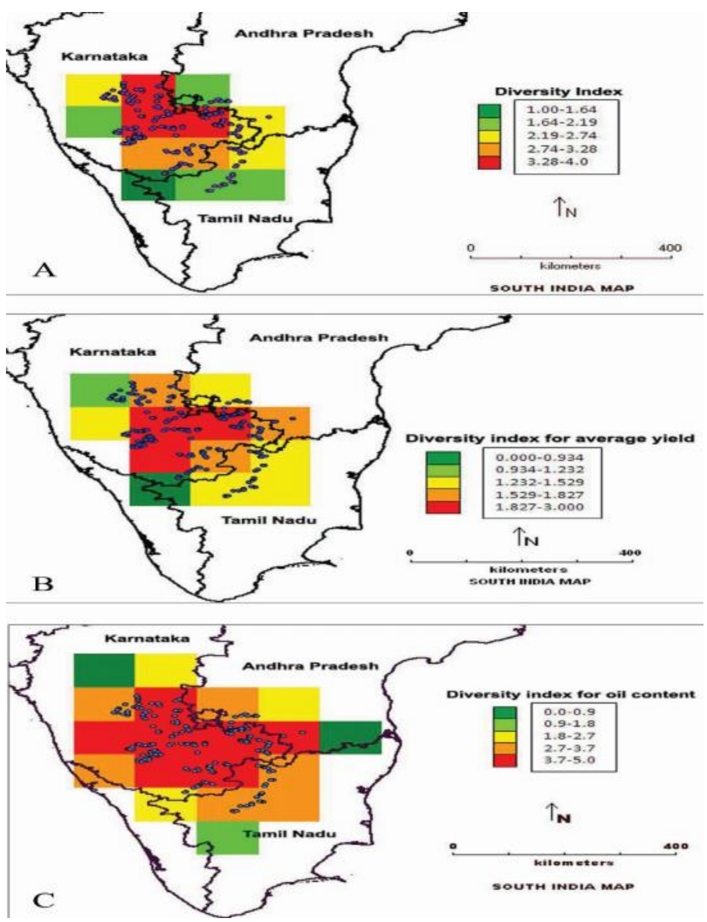

FIGURE 3. DIVA-GIS grid map showing the (A) diversity index for *Pongamia pinnata* germplasm (B) diversity index for average seed yield (C) diversity index for seed oil content collected from different agro-ecological zones of the study region.

A high diversity index for average seed yield per plant (Fig. 3B) was observed in few regions of central dry zone, eastern dry zone and southern dry transition zone of Karnataka followed by transition zone between Karnataka and Andhra Pradesh and in a few provenances of central dry zone. The accessions of Tamil Nadu showed low diversity index for average seed yield per tree. Low diversity index for oil content was observed in regions of Tamil Nadu and Andhra Pradesh, while high diversity index for oil content (Fig. 3C) was recorded in the agro-ecological zones of Karnataka. This study indicated that GIS could be used effectively to assess the pongamia diversity and identify regions with high density trees. The grid maps revealed that provenances of Chitradurga, Kolar, Hassan, Tumkur, Bengaluru rural, Davanagere and Chamarajnagar were promising locations for the presence of diverse pongamia genotypes with regard to oil content. Diverse genotypes with regard to average seed yield were recorded from former three provenances as well as in some parts of Tumkur. DIVA-GIS application was employed to predict the diversity and distribution of Jatropha species in south east coastal zone of India (Sunil *et al.,* 2009). Attempts have also been made in other plant species to predict various concepts of diversity, by DIVA-GIS (Miller and Knouft, 2006). Varaprasad *et al.,* (2008) employed DIVA-GIS for assessing the diversity of fatty acid composition in linseed from peninsular India. The present survey revealed that the distribution of pongamia is diverse across all the selected agro-ecological regions and this enables us to predict the gaps in the collection and richness of genotypes. Further, a detailed documentation of the germplasm availability based on field survey, climatic and rainfall patterns could be employed to determine pongamia distribution in zones identified in Karnataka, Andhra Pradesh and Tamil Nadu. This type of study help in generating the base line data for further analysis related to the exploration, conservation and use of pongamia germplasm for the identification of quality planting material, developing clonal orchards with high oil content in seeds for biodiesel production. The GIS mapping is an excellent tool and a pre-requisite step towards documentation, identification of the gaps in the germplasm collection, preliminary diversity analysis, assessment of loss in the density of germplasm, development of conservation methods and sustainable utilization of the germplasm and prediction of the distribution of genotypes based on the altitude and rainfall pattern (Hijmans and David, 2001).

Effect of environmental variables on morphological traits

The CCA ordination biplot (Table 4) indicated the contribution of altitude and soil on important morphometric traits like plant height, GBH, number of main branches and sub-branches, branching pattern, fruits per bunch, average seed yield and oil content (Fig. 4). The ordination diagram pointed out at the distribution of morphometric traits along each environmental variable. The effect of altitude on morphometric traits has been reported in legume species like *Pueraria mirifica* (Suwanvijitr *et al.,* 2010). The length and direction of vector representing altitude and soil showed a direct correlation between the fruits per bunch and average seed yield (Fig. 4). Similar studies on direct effect of high elevation on seed yield have been reported in the oilseed species of *Lesquerella* of Brassicaceae (Dierig *et al.,* 2006). The plant height, GBH, number of main branches and sub-branches, branching pattern and oil yield negatively correlated with altitude and soil factors (Table 4). The above results suggested that the high seed yield depended on the environmental variables. Manian and Gopalakrishnan (1995) reported the influence of altitude on oil yield of *Eucalyptus globules.* However, in case of *Jatropha curcas*, altitudinal variations had no effect on oil content (Pant, 2006).

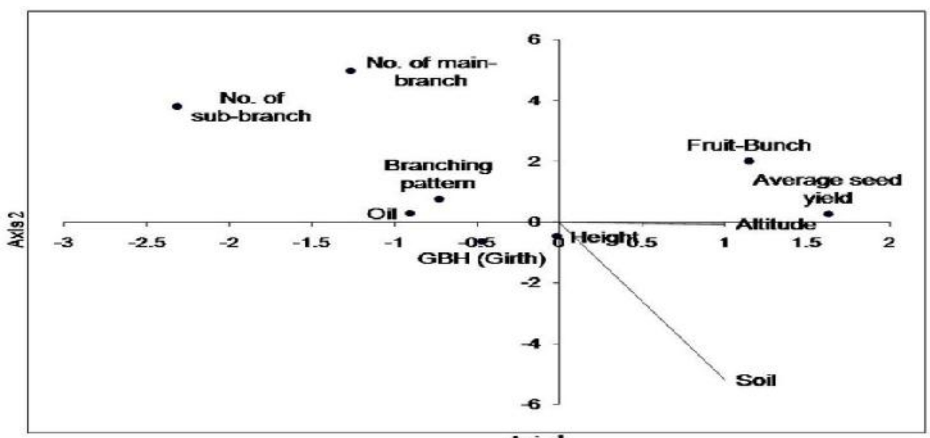

FIGURE 4. Canonical correspondence analysis ordination biplot of the morphometric traits of *Pongamia pinnata* CPT scores with environmental variables prevailing in different agro-ecological zones of the study region

Clustering based on morphometric study

Based on the Euclidean distances and UPGMA, 232 CPT were grouped into two main clusters I and II (Fig. 5). These clusters were further subdivided into four sub-clusters- A, B, C and D. The main cluster I included the CPT with beaked pod tip and the main cluster II included CPT with mucronate pod tip. Sub-cluster A consisted of 7 CPT, while the sub-cluster B formed a major cluster with 93 CPT, followed by sub-cluster C consisting of 71 CPT and sub-cluster D with 61 CPT (Table 5). Most of the CPT with erect branching pattern was grouped together in the sub-cluster D.

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TABLE 2: Morphometric and yield characterstics of *Pongamia pinnata* germplasm from different agro-ecological zones of southern peninsular India

Traits	Classification	Study regions for collection/ Number of germplasm observed				
		Central dry zone of Karnataka (Zone 1)	Eastern dry zone of Karnataka (Zone 2)	Southern dry and transition zone of Karnataka (Zone 3)	Transtion zone between Karnataka and Andhra Pradesh (zone 4)	Transtion zone between Karnataka and Tamil Nadu (Zone 5)
		50	84	57	12	29
Soil type	Black	6	4	6	2	8
	Red	40	77	51	10	21
	Red rocky	4	3	–	–	–
Tree age (yr)	<10	3	–	4	–	–
	10-20	21	17	19	2	9
	20-30	9	20	11	4	10
	30-40	14	20	8	2	6
	40-50	2	18	10	3	2
	>50	1	9	5	1	2
Height (m)	<3	–	–	–	–	–
	3-6	20	11	10	3	8
	6-9	17	34	19	6	14
	9-12	10	21	23	3	5
	12-15	2	13	4	–	2
	>15	1	5	1	–	–
GBH (cm)	≤20	21	1	–	–	–
	30-50	15	9	12	4	2
	50-70	1	6	5	2	2
	70-90	5	5	8	4	2
	>100	8	63	32	2	23
Main branches (no.)	≤3	43	76	53	12	25
	≥4	7	8	4	–	4
Sub branches (no.)	<5	37	14	33	12	10
	5-10	13	68	24	–	19
	>10	–	2	–	–	–
Canopy diameter (m)	≤3	1	1	–	–	–
	3-7	30	28	25	9	14
	7-15	19	47	31	3	15
	>15	–	8	1	–	–
Canopy shape	circular	40	67	31	10	17
	conical	10	17	26	2	12
Branching pattern	Drooping	39	72	42	10	17
	Erect	11	12	15	2	12
Fruits per bunch (no.)	<10	30	21	35	11	28
	10-15	19	43	15	1	1
	>15	1	20	7	–	–

Pod texture	Smooth	30	34	19	9	20
	Rough	20	50	38	3	9
Pod tip	Mucronate	31	50	31	5	19
	Beaked	19	34	26	7	10
Pod size (mm)	Small (30 - 35mm)	16	24	13	4	15
	Medium (40 - 50mm)	33	56	36	8	13
	Large (55 -75mm)	1	4	8	–	1
Pod thickness (mm)	Bulged (10 -15mm)	17	61	26	3	12
	Flat (5 -10mm)	33	23	31	9	17
Average seed yield (kg / tree)	20-40	20	9	13	9	21
	40-60	24	23	23	3	7
	>60	6	52	21	–	1
Oil content (%)	<30	8	7	10	4	7
	30-35	32	46	38	6	22
	35-40	10	30	8	2	–
	>40	–	1	1	–	–

TABLE 3: Zonal variations in morphometric traits of *Pongamia pinnata* germplasm

Agro-ecological zones	Height (m)	GBH (cm)	Main branches (no.)	Sub-branches (no.)	Fruits per bunch	Average seed yield (kg)	Oil content (%)
Central dry Zone of Karnataka (zone 1)	9.65^{ab}	103.00^{ab}	4.00^{a}	8.84^{a}	8.82^{ab}	53.34^{ab}	33.63^{b}
Eastern dry zone of Karnataka (zone 2)	12.44^{a}	155.00^{ab}	3.1^{ab}	7.34^{ab}	12.54^{a}	61.67^{a}	34.86^{a}
Southern dry and transition zone of Karnataka (zone 3)	9.4^{ab}	147.7^{ab}	3.17^{ab}	4.84^{b}	9.65^{ab}	55.01^{ab}	33.13^{c}
Transition zone between Karnataka and Andhra Pradesh (zone 4)	7.62^{b}	65.84^{b}	2.67^{b}	7.01^{ab}	8.09^{b}	38.34^{b}	32.46^{d}
Transition zone between Karnataka and Tamil Nadu (zone 5)	9.14^{ab}	170.7^{a}	3.34^{ab}	6.84^{ab}	7.76^{b}	46.67^{ab}	32.20^{e}
LSD (P = 0.05)	3.22	93.73	0.92	2.97	3.65	17.44	0.96

Data is the mean of values observed in CPT marked in each agro-ecological zone. Mean values that are followed by same letters are not significant according to DMRT test; LSD= Least Significant Difference

TABLE 4: Eigen values, total variance, correlation values and ordination scores for the canonical correspondance analysis (CCA) of *Pongamia pinnata* CPT from different agro-ecological zones

Category	Axis1	Axis2	Axis3
Eigen value	0.003	0.002	0.068
Total variance	0.0976		
% of variance explained	3.4	2.2	69.5
Cumulative % explained	3.4	5.6	75.2
Pearson Correlation (morphometric traits-Environment)	0.384	0.262	0.000
Kendall Correlation (morphometric traits-Environment)	0.257	-0.024	0.000
Ordination Scores for environmental variables			
Altitude	0.057	-0.004	0.000
Soil	0.009	-0.046	0.000

TABLE 5: Classification of CPT based on UPGMA cluster analysis and squared euclidean distance for morphometric traits in *Pongamia pinnata*

Cluster	Number of Accessions	Accessions
Cluster A	7	CPT 104, CPT 184, CPT 144, CPT 13, CPT 4, CPT 50, CPT 120
Cluster B	93	CPT 16, CPT 25, CPT 22, CPT 14, CPT 27, CPT 28, CPT 31, CPT 20, CPT 35, CPT 33, CPT 29, CPT 32, CPT 37, CPT 38, CPT 34, CPT 50, CPT 48, CPT 43, CPT 58, CPT 71, CPT 55, CPT 59, CPT 40, CPT 56, CPT 81, CPT 80, CPT 78, CPT 84, CPT 85, CPT 79, CPT 87, CPT 92, CPT 105, CPT 134, CPT 103, CPT 6, CPT 98, CPT 108, CPT 102, CPT 109, CPT 15, CPT 112, CPT 107, CPT 126, CPT 110, CPT 125, CPT 122, CPT 121, CPT 124, CPT 111, CPT 123, CPT 127, CPT 155, CPT 145, CPT 136, CPT 143, CPT 8, CPT 86, CPT 146, CPT 147, CPT 152, CPT 128,CPT 158, CPT 159, CPT 165,CPT 179,CPT 169, CPT 182, CPT 173, CPT 168, CPT 171, CPT 12, CPT 185, CPT 188, CPT 190, CPT 187, CPT 200, CPT 193, CPT 194, CPT 197, CPT 218, CPT 192, CPT 202, CPT 228, CPT 214, CPT 199, CPT 222, CPT 232, CPT 227, CPT 209, CPT 229, CPT 231, CPT 226
Cluster C	71	CPT 10, CPT 2, CPT 3, CPT 11, CPT 7, CPT 9, CPT 1, CPT 5, CPT 17, CPT 18, CPT 19, CPT 138, CPT 23, CPT 24, CPT 26, CPT 41, CPT 36, CPT 46, CPT 30, CPT 42, CPT 45, CPT 52, CPT 39, CPT 47, CPT 49, CPT 51, CPT 44, CPT 62, CPT 54, CPT 57, CPT 180, CPT 61, CPT 53, CPT 66, CPT 60, CPT 65, CPT 63, CPT 73, CPT 70, CPT 69, CPT 75, CPT 72, CPT 219, CPT 90, CPT 68, CPT 88, CPT 77, CPT 74, CPT 83, CPT 76, CPT 91, CPT 82, CPT 89, CPT 97, CPT 94, CPT 95, CPT 100, CPT 93, CPT 99, CPT 96, CPT 101, CPT 106, CPT 118, CPT 114, CPT 116, CPT 119, CPT 113, CPT 137, CPT 129, CPT 133, CPT 117
Cluster D	61	CPT 132, CPT 130, CPT 135, CPT 131, CPT 21, CPT 139, CPT 140, CPT 141, CPT 153, CPT 148, CPT 149, CPT 150, CPT 151, CPT 142, CPT 224, CPT 156, CPT 157, CPT 160, CPT 161, CPT 162, CPT 163, CPT 164, CPT 216, CPT 167, CPT 170, CPT 172, CPT 174, CPT 175, CPT 176, CPT 177, CPT 178, CPT 64, CPT 181, CPT 183, CPT 186, CPT 189, CPT 191, CPT 195, CPT 196, CPT 198, CPT 201, CPT 203, CPT 204, CPT 205, CPT 206, CPT 207, CPT 223, CPT 210, CPT 211, CPT 212, CPT 213, CPT 215, CPT 166, CPT 217, CPT 230, CPT 220, CPT 221, CPT 208, CPT 154, CPT 225, CPT 67

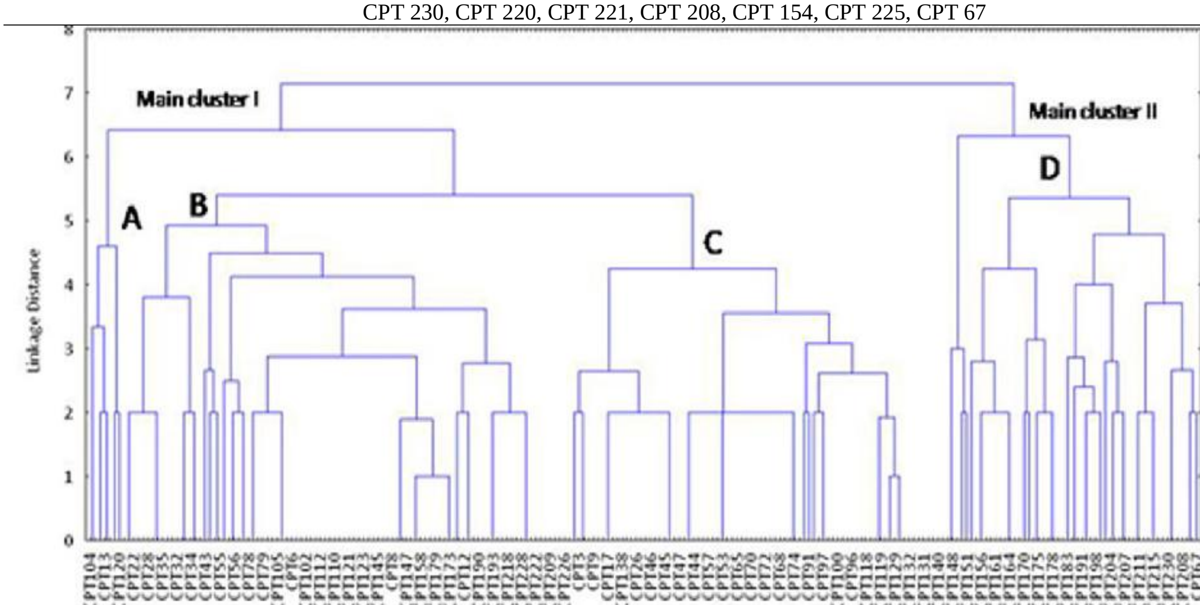

FIGURE 5. Dendrogram constructed based on the morphometric traits of 232 CPT of *Pongamia pinnata* collected from different agro-ecological zones.

(A, B, C, D indicates the sub-clusters. CPT representing these clusters has been given in table 5)

The CPT with large pod size was grouped together in sub-cluster A, while the CPT with small pod size were clustered together under sub-cluster D. The CPT with conical canopy was merged together in sub-cluster D. This indicated that the conical trees with erect branches yielded small pods. Euclidean distance ranged from 1 to 12. The CPT 104 of Tumkur provenance of zone 2 showed highest Euclidean distance (12) from CPT 67 of Kolar provenance of zone 2. These CPT from the same agro-ecological zone have been grouped apart in the dendrogram. The random grouping of CPT from different agro-ecological zones for the qualitative morphometric traits yielded several clusters. Such clustering has been reported in safflower genotypes from different agro-ecological zones of Iran by Amini *et al.*, (2008). The results of cluster analysis based on qualitative morphometric traits suggested that some CPT with similar geographical area were clustered into different groups and there were no expectable relationship between patterns of dendrogram and agro-ecological zones. Khan *et al.* (2009) reported similar kind of clustering in safflower with no correlation between geographical location and morphological traits. Similar findings, based on the quantitative and qualitative traits, showing no specific pattern of clustering was reported in the *Tamarindus indica* genotypes from Devanahalli taluk (Nandini *et al.*, 2011). Based on the cluster analysis, the selection of CPT for seedling production could be taken up from pongamia trees having circular canopy with drooping branching pattern.

CONCLUSION

It could be concluded from the present investigation that *Pongamia pinnata* is distributed widely across the central dry zones and eastern dry zones of Karnataka as compared to the other selected agro-ecological zones. DIVA-GIS approaches could be used to assess pongamia diversity in other regions of India, other countries to identify the regions with high tree density. The selection of elite germplasm from these regions of Karnataka could be considered for the breeding program since there existed a large population with diversity. The high genetic variability existing within the natural populations of pongamia in the study regions could be exploited by the tree breeders for the sustainable development of biofuel industry in India and other developing countries. Further, improvement of pongamia genotypes for biofuel purpose is shown to involve in the exploration of population, collection of distinct germplasm, evaluation of yield characteristics, multiplication, conservation and maintenance of germplasm. Such of the genotypes with superior quantitative traits could be used for mass multiplication by conventional and micro-propagation methods and for energy plantations in wastelands.

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