General Discussion
The review of literature revealed that there exists a few reports on the genetic variability and divergence studies in *Pongamia pinnata* in Haryana-40 CPT (Kaushik et al., 2007b), Andhra Pradesh and Orissa -123 CPT (Sunil et al., 2009a), Jharkhand-24 CPT (Divakara et al., 2010), Andhra Pradesh -50 CPT (Rao et al., 2011), Maharashtra-20 CPT (Raut et al., 2011) and northern Karnataka (Patil et al., 2011). Pongamia grows well in arid and semi-arid regions and this condition prevails in the peninsular India. A preliminary survey indicated that pongamia trees in these regions have variation in several morphological characteristics. However, these studies are restricted to narrow geographical areas with less diverse populations. Therefore, the present study was taken up to document the distribution of pongamia germplasm across different agro-ecological zones of southern peninsular India. Studies were conducted to collect and evaluate pods and seeds for their characteristics and also study the genetic diversity and biochemical traits and identify hot-spots of trees capable of producing oil with biofuel property.

Pongamia is a widely distributed biofuel species with a considerable genetic variation in morphological, biochemical and genetic diversity. The study area involving the selected agro-ecological zones of the southern peninsular India in which the CPT were selected was governed by the occurrence of vast pongamia population. Two hundred and thirty two accessions were selected for studying the morphometric diversity in five agro-ecological zones from the study area - the central dry zone of Karnataka state (zone 1), eastern dry zone of Karnataka state (zone 2), southern dry and transition zone of Karnataka state (zone 3), transition zone between Karnataka and Andhra Pradesh (zone 214...
Cf'eneraf (Discussion 4) and transition zone between Karnataka and Tamil Nadu (zone 5). The pongamia population was widely distributed in the central dry and eastern dry zones of Karnataka when compared to other zones. Davanagere, Chitradurga, Kolar, Tumkur, Bengaluru Rural, Hassan and Chamarajanagar were identified as potential source for CPT with high seed yield and oil content. The morphological traits are considered important in predicting the yield of a plant (Richard, 2006). The CPT with superior phenotypic characters like drooping branches, 5-10 sub-branches, large canopy, 10-15 fruits per bunch, high seed yield and 33-38% seed oil content were considered as seed source for biofuel program, mass propagation and breeding.

The zonal variation in morphological traits could be attributed to annual rainfall, temperature and soil type. The altitude and soil directly correlated with fruits per bunch and average seed yield. The altitude also showed positive correlation with 100 pod weight and negatively correlated with oil content. The CPT from southern dry and transition zone of Karnataka performed better for pod and seed traits. Similar kind of zonal variation with respect to morphology and oil content has been reported in oilseeds (Kaura et al., 1998; Mohapatra and Panda, 2010). The CPT from Hassan provenance showed high 100 pod weight, 100 seed weight, length and breadth of pod and seed due to prevalence of local climatic conditions. The seed morphology plays an important role in seed germination and survival of seedlings in pongamia (Manonmani et al., 1996). In many tropical tree species, decline in seed weight with respect to latitude has been reported (Moles and Westoby, 2003). similar results were also observed in pongamia.

The PCV value was higher than GCV value for pod, seed and biochemical traits indicating the influence of environmental variables. The PCV value was high for 100
seed weight indicating that the storage of food reserves during seed development could be influenced by the environment (Luzuriaga et al., 2006). High heritability and genetic advance for 100 seed weight, seed-pod ratio was observed for zone 1 and accessions from zone 2 for seed germination indicating that CPT from these zones could be selected as superior seed source. The above observations for 100 pod and seed weight are in accordance with the studies on pongamia from Haryana (Kaushik et al., 2007b), Jharkhand (Divakara et al., 2010), Andhra Pradesh and Orissa (Sunil et al., 2009a). High heritability along with genetic advance for desired traits are useful in the selection of seed source for breeding (Volker et al., 1990). Both correlation and path analysis studies showed that seed weight and thickness was directly correlated to oil content. Therefore, the seed weight and thickness could be identified as an important trait in the identification of superior genotypes with high seed yield and oil content.

The phenotypic traits are largely influenced by the environmental variables. Therefore, the use of seed proteins, DNA markers and biochemical markers are recommended which provide unbiased results on diversity of a species. In the present study, the zone-specific protein bands were identified at 29 and 43 kDa that could be used for the identification of unknown trees from different dry zones. In recent years, AFLP marker system has gained extensive importance in genetic diversity analysis (Elias et al., 2000). There are a few reports on the genetic divergence of pongamia population in terms of PCR-based markers in north Guwahati (Kesari et al., 2010b) and Delhi (Sharma et al., 2010) with each restricted to narrow geographical area. Thudi et al. (2010) studied the genetic diversity using the AFLP markers across different states of India, including a few locations of the present study region. They also showed the effectiveness of five primer
combinations to determine the diversity of pongamia. The above primers were found to be less effective when examined in this study. The primer combination of E-ACG/M-GCC showed highest polymorphism of 98.8%. The primer combinations E-ACG/M-GCC, E-CAC/M-CTA with high PIC, MI and RP could be used for further studies on genetic diversity analysis in pongamia. In the present study, unique bands including accession-, zone- and provenance-specific bands were identified and these could be used for further screening of accessions. Since pongamia is cross pollinated, the variance level was high within the provenance when compared to variation among agro-ecological zones and provenance. Similar observations were observed in tropical legumes (Cardoso et al., 1998; Rivera-Ocasio et al., 2002). The genetic differentiation among pongamia populations could be due to their exposure to various selection pressures in their natural habitat.

The biochemical profiling of the CPT showed significant variation with respect to different agro-ecological zones. The quality and quantity of pongamia seed oil from the studied CPT was found to be on par with the previous reports (Scott et al., 2008). The CPT from these zones 1, 2, 3 and 4 were identified as potential seed source based on their high heritability for oleic acid. El-Beltagi and Mohamed (2010) reported that the reduced content of linoleic and linolenic acid and increased oleic acid content resulted in good quality biodiesel. The biochemical traits showed association with environmental variables and among themselves. This variation could be due to the enzyme activity, environmental variables, gene action (Chiou et al., 1995; Singkham et al., 2012). The divergence study of genotypes helps to understand the effect of morphological traits, geographical location,
genetic and biochemical traits on the pattern of clustering. The highly divergent trees are preferred for breeding program (Sunil et al., 2009a).

The clustering pattern based on morphological traits indicated that seeds could be collected from trees with circular and drooping branching pattern. The heterogeneous clustering pattern was observed for qualitative morphological traits, as well as pod and seed traits which depended on the geographical locations. On the other hand, a homogeneous clustering pattern was observed for protein, genetic and biochemical traits depending on the geographical location. This pattern of clustering could have resulted due to the selection of some of the representative CPT as against 232 CPT selected for morphological traits. The other probable reason could be due to the migration of certain CPT to other areas or due to deliberate exchange of germplasm. Therefore, 33 CPT selected for cluster analysis of protein, genetic and biochemical traits were considered for comparative analysis. Hence, those 33 CPT that were considered for clustering analysis of protein, genetic and biochemical traits were also determined for their clustering behavior with respect to morphological traits like 100 pod weight, seed oil and oleic acid content. A comparative analysis of the clustering pattern of the above characteristics indicated that the CPT from central dry zone (Davanagere and Chitradurga provenances) and eastern dry zone (Tumkur, Bengaluru rural and Kolar provenances) separated distinctly for all the characteristics like 100 pod weight, high oil and oleic acid content, seed protein and AFLP markers. Based on the above, traits like 100 pod weight, oil and oleic acid content could be identified not only by morphological traits but also by biochemical and molecular markers.
Pongamia and jatropha are being promoted as sources of biofuel in India. Of late much importance is given to pongamia since it is widely distributed across India with less crop management practices and high seed yield when compared to other non-edible oilseeds. Oleic acid is the desired fatty acid content for biodiesel production (Knothe, 2002). Review of literature revealed that the oleic acid content in non-edible oilseeds like *P. pinnata* (46-65%), *A. indica* (25-58%), *M. longifolia* (32-48%), *J. curcas* (42-48%) and *C. inophyllum* (39-50%) are suitable for biodiesel production. Results of the present study confirmed that pongamia oil is highly suitable for biodiesel production with high monounsaturated fatty acid and low polyunsaturated fatty acid. The ratio of saturated to unsaturated fatty acid also determines the quality of biodiesel and this ratio depended upon the pongamia seed development. In many oilseeds, it is reported that the ratio of saturation to unsaturation in oil is dependent upon the harvest time (Haathurusingha *et al.*, 2010).

In legumes and non-legumes, many storage reserve compounds along with fatty acids accumulate at different time intervals during seed development. A preliminary review of literature indicated the study on seed maturation stages in pongamia growing in Guwahati mainly based on seed length, width and thickness, total seed protein and ultrastructural changes (Kesari and Rangan, 2011). However, this study provided less information regarding the histological, histochemical, biochemical changes and harvest time of mature pongamia seed. Therefore, the present study was taken up to document the biochemistry of seed development. The pongamia fruit development (30 to 42 WAF) was marked by early green pod stage, middle half brown stage and late dark brown stage. Significant variation in seed biomass, pod and seed traits, and moisture content was
observed suggesting a major physical and physiological change during development. The
general structure of pongamia seed during the development corresponded to that of other
legumes (Miller et al., 1999). The major component of pongamia seed coat was found to
be lignin, while cotyledon was made up of polysaccharides, proteins and starch granules.

The oil and oleic acid content increased during 30 to 42 WAF coinciding with the
simultaneous increase in oil bodies (200 to 300 per cell) at 42 WAF. Palmitic and linoleic
acid decreased, while stearic acid and linoleic acid remained constant during the seed
maturity. The localization of seed storage reserves in pongamia was similar to other
legumes (Coelho and Benedito, 2008). The qualitative properties of oil, like iodine value
and saponification value at different developmental stages showed marked changes
suggesting the importance of harvest time of pongamia seed for biodiesel production. The
variation in saturation to unsaturation level in oil could be attributed to the enzymes
involved in fatty acid biosynthesis during seed development (Wu et al., 2009). The
carbon and nitrogen plays an important role during legume seed development (Weber
et al., 2005). In the present study, the variation in carbon and nitrogen along with other
mineral nutrients was observed which coincided with the variation in seed dry biomass,
amino acid and protein content at 42 WAF. At the later stages of development,
chlorophyll content was found to decrease, since abscisic acid induced the degradation of
chlorophyll during seed desiccation (Nakajima et al., 2012). The low karanjin content at
the initial stages of seed development could be ideal for biodiesel production. Since,
karanjin is an value addition to pongamia oil the extraction of karanjin for industrial
purpose could be taken up at later stages of seed development.
Results of the present study have shown that premature harvesting of pods resulted in the reduced oil content, fatty acids and other storage reserves. Therefore, it is recommended to harvest pods and extract the oil at the end of 42 WAF for the production of good quality biodiesel. The present study helped in the generation of base line data for seed collection from various agro-ecological zones of southern peninsular India for the purpose of mass multiplication by conventional and micro-propagation methods and for energy plantations on wastelands. The above observations also help in the building up of database for further studies involving the characterization of proteins and enzymes in the biosynthetic pathway of oil.