Tamarindus indica L. is an economically important multipurpose tree species, which is grown both as domesticated in farmland and wild in forest wastelands. In the management of these economically viable trees, emphasis is more on conservation of the plus trees and ensuring sustained yields, (Gangaprasad 1993). There is a considerable demand for fruit pulp, seed and seed powder. Hence to cope up with the large demand, it has become necessary to identify superior elite trees for monoculture plantations, without causing genetic erosion. Establishing plantations using genetically improved clones will help to maximize production. Evaluation of the germplasm already present also plays equal importance. This will lead to indirect economic and social benefits to dry land farmers.

High yielding and regular bearing trees can be identified by the evaluation of elite genotypes. Genetic information can be utilized for selection of superior genotypes among Tamarindus indica L. populations. Selection can be made based on the markers linked to useful traits and can be conserved ex-situ through vegetative propagation. The uses of such markers not only facilitate the selection process but also provide information on existing elite genotypes and can be used as descriptors for documentation. Despite the economic and agricultural value of Tamarindus indica L., no attempts have been made to utilize genetic markers for tree improvement. Molecular tools/techniques can be used as diagnostic tools to assist the conventional
breeding process and for selections existing among the natural genotypes evolved randomly due to cross-pollination.

Since the present work is on Genetic diversity, assessment at morphological, molecular and biochemical levels provides more procure estimation of genetic diversity. Research work in these areas pertaining to *Tamarindus indica* L. and other tree species is limited to understand the procedures and interpretation of Genetic diversity assessment work done in other crops were also revived wherever necessary.

2.1 PHENOTYPIC DIVERSITY

Tree improvement through the application of genetic principles is basically directed towards modifying the heredity of tree population, so that trees are able to meet the needs of the grower. The knowledge of the breeding systems and the inheritance of pulp yield and associated characters are necessary in evaluation of existing germplasm. Phenotypic studies mainly concentrate on variability, multivariate and correlation studies. Similar kind of studies on this species can be seen from the works of Gangaprasad (1993) and Hanamashetti (1996).
2.1.1 Variability Studies

2.1.1.1 Tamarindus indica L.

According to Paules (1975), Tamarindus indica L. is mostly grown as self sown or by sowing seeds of unknown parentage, which has resulted in wide variation among seeding progenies. It is very desirable if proper selection of mother plants is made for the collections of seeds for planting purpose.

Thimmaraju et al., (1978) reported that Tamarindus indica L. is a highly cross pollinated tree and offers scope for selection of superior clones.

Samiullah (1984) assessed the variability in Tamarindus indica L. across 300 genotypes and noticed wide variability for all the characters studied. The variations observed for the characters, viz., trunk length, trunk volume, pod yield, pulp yield, seed yield, etc., were significant.

Gangaprasad (1993) studied about two hundred and eighty two trees of Tamarindus indica L. from eight provenances of six districts, viz., Shimoga, Chitradurga, Bengaluru, Tumkur, Mandya and Kolar. Wide variation was observed for the characters like trunk volume, pod weight, number of seeds per pod, seed weight per pod, seed to pod ratio, pod yield, seed yield, pulp yield and tartaric acid yield per tree and number of primary branches per tree. The success of genetic improvement in tree species like Tamarindus indica L. depends to a large extent on the assessment of variability for quantitative
Chapter 2 - Review of Literature

characters, identification of divergent plus trees, mass multiplication of elite trees and their proper conservation.

The studies conducted by Hanamashetti (1996) on evaluation of 40 genotypes of *Tamarindus indica* L. in Northern Karnataka also showed significant variations for different characters viz., plant height, diameter, number of shoots per 900 cm² area, spread of plant East-West, North-South and Crown size.

2.1.1.2 Other Tree Species

Clonal variation for growth and morphological traits was observed in *Populus deltoids* (Ying and Bagley, 1976). Jha *et al.*, (1991) reported that when data on poplar clones (*Populus deltoids* and *P. xeuramericana*) both exotic and indigenous were subjected to statistical analysis at half the rotation age the variation in height was insignificant, while in diameter and survival it was significant.

The variability study in *Eucalyptus camaldulensis* showed significant variation for eight morphological characters of leaves (Burley *et al.* 1977). A one per cent progeny test in *E.grandis* was assessed for plant height at four different ages (Kedharnath, 1982).

It was noticed that there was a large magnitude of genetic variation in mean plant height between the families. Kapur and Dogra (1987) showed that...
provenances of *E. camaldulensis* and *E. tereticornis* varied significantly for the growth parameters viz., height, diameter and volume.

Manaturagimath *et al.*, (1991) observed significant variation in *E. cloeziana* provenances for survival percentage, height, diameter at breast height (dbh) volume and mean annual increment.

The genetic variances among nine provenances of *E. tereticornis* were not significant at an age of three years. Just detectable (at the ten per cent level of probability) at an age of five years and highly significant (at one per cent level of Probability) at an age six years (Otegbeye, 1991).

*Tectona grandis* (Teak) differed significantly for traits, height and diameter at breast height (dbh) in provenance according to Suri (1984) and Krishnamoorthy (1989).

Kumaran (1991) found significant variation between 28 (one per cent) families of *Pongamia pinnata* in seed parameters and seedling traits. Study also showed the genotypic coefficients of variation for basal diameter and volume were 21.82 and 29.74 respectively.

Singh and Chaudhary (1992) obtained maximum variation for plant height followed by number of branches, base diameter, leaf breadth and leaf length among 28 families of *Prunus armeniaca*. 14
In *Bambusa balcooa*, Singh and Beniwal (1993) found that genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for plant height and number of new shoots were maximum at the age of four and half years and three and half years and minimum at the age of two and half years.

Srivastava *et al.*, (1993a) observed that eight years old *Terminalia arjuna* recorded the highest genotypic coefficient of variation for leaf yield followed by length of leaf and number of leaves per branch. On the contrary, maximum phenotypic coefficient of variation in leaf yield was followed by breadth of leaf, number of branches per plant and number of leaves per branch. The GCV values were lower in magnitude than PCV in all the seven characters studied except for length of leaf revealing that environment greatly influenced expression of these characters.

Half-sib seedlings belonging to 24 *Terminalia arjuna* trees showed high genetic coefficient of variation for seedling height indicating additive gene action for this (Srivastava *et al.*, 1993b).

### 2.1.2 Correlation Studies and Path Coefficient Analysis

Correlation coefficient analysis helps to determine the nature and degree of relationship between any two measurable characters. It resolves the complex relations between important characters, which are of immense help in the selection of suitable clones. But a measure of correlation does not
consider the dependence of one variable on the other. The direct contribution of each component to the yield and the indirect effect it has through its association with other components cannot be differentiated from mere correlation studies. A statistical device called the path coefficient analysis developed by Wright (1921) fulfills this lacuna. It is a tool in genetic analysis to partition the association of the components on yield and indirect effect of the characters through other components.

The path coefficient analysis is useful to study the cause and effect relationship, diagrammatically. However, such studies in tree species are scarce.

2.1.2.1 Tamarindus indica L.

Samiullah et al., (1993) in a study involving 300 genotypes of Tamarindus indica L. identified that characters like number of primary branches (0.459) length of pod (0.448), pulp yield per tree (0.712), trunk volume (0.816), seed yield (0.848) and tartaric acid (0.848), showed strong positive and significant association with pod yield. The path coefficient analysis of yield contributing characters revealed that pulp yield per tree and seed yield per tree had appreciable direct effect.

Gangaprasad (1993) showed correlation and path coefficient among the three regions across provenances of southern Karnataka. The direct contribution to pulp yield per tree by pod yield per tree was the highest
followed by pulp to pod ratio and pulp weight per pod among the genotypes from Chitradurga I. Genotypes from Bangalore showed the direct contribution of pod yield per tree was highest towards pulp yield per tree, followed by pulp weight per pod and seed yield per tree and direct effects of pod length and seed weight per pod were negative. Genotypes from Kolar II showed direct effect on pulp yield per tree by pulp to pod ratio was the highest followed by pod yield per tree and pod width.

Hanamashetti (1996) showed the degree of correlation among the genotypes studied with respect to growth parameters in *Tamarindus indica* L. Plant height and diameter were positively and significantly correlated with plant spread, (east – west and north – south) and crown size. Number of pods and pod weight were positively associated with crown size.

### 2.1.2.2 Other Tree Species

Kedharnath *et al.*, (1969) reported that in *Tectona grandis* (teak) the genetic correlation of 0.90 was observed between stem girth and number of internodes. Also Lakshmikanthan *et al.*, (1974) reported high positive correlation between height and diameter.

Khosla *et al.*, (1980) opined that there was a strong correlation between height and diameter and ratio of clear bole to total height in *Populus ciliata*. A strong negative genetic correlation of green house height with second year height was noticed by Nelson and Tauer (1987) in *P. deltoides*. 

17
Casuarina equisetifolia recorded a significant and positive correlation between total biomass and other traits like girth at breast height, stem weight and volume in study by Stephan Durairaj (1981) and Jambulingam (1989). Further, opined that maximum positive direct effect on wood yield was exerted by girth at breast height followed by total height, merchantable height, girth at stump level and weight of bark in that order in Eucalyptus tereticornis while bark thickness exerted negative indirect effect on yield.

Rathinam et al., (1981) observed a strong positive correlation between total height, girth at breast height and weight of green bark in Eucalyptus tereticornis. Further, opined that maximum positive direct effect on weed yield was exerted by girth at breast height followed by total height, merchantable height, girth at stump level and weight of bark in that order in Eucalyptus tereticornis while bark thickness exerted negative indirect effect on yield.

Volker et al., (1990) in Eucalyptus globules reported a strong genetic (0.82) and phenotypic (0.66) correlation between height and stem diameter. Volume showed very high genetic correlations with both height and diameter. The same trend was apparent in the phenotypic correlations between them.

Sukumaran et al., (1982) studied path coefficient analysis for 13 traits on 43 Progenies of eight high yielding west coast tall coconuts planted in 1953. Analysis of the data indicated that average number of female flowers at 21 to
24 years, number of functional leaves at 19 years and internodal distance at a fixed mark had the greatest direct effects on average yield at 21 to 24 years.

Ramanathan (1984) conducted correlation studies in 30 cultivars of coconut which were 23 years old and noticed significant and positive correlation of stem height \( r = 0.623 \) with nut yield.

Lebeder and Chucha (1984) noticed that leaf area per rosette in apple was an important character associated positively with yield and yield pattern of fruit bearing. The correlations found were of considerable use in selecting promising parents for yield.

Magdalita et al., (1984) studied character associations in 100 accessions of papaya and study revealed that fruit weight was positively and significantly correlated with fruit length, width, flesh thickness and cavity volume.

Kumaran (1991) suggested height as the reliable character for selection in *Azadirachta indica* (neem) as it exhibited maximum correlation, both phenotypic and genotypic, for sturdiness quotient. Further opined that, in *Azadirachta indica* (neem) shoot length recorded the maximum positive direct effect followed by volume index, number of branches, and root length.
Kumaran (1991) reported that height exhibited maximum positive genotypic and phenotypic correlation with sturdiness quotient in *Pongamia pinnata*. The study revealed that height could be the reliable character for selection.

The correlation studies in *Dalbergia sissoo* (Dhillon et al., 1992) revealed that diameter (dbh) showed positive and highly significant association with height of crown as well as total height. Further opined that, out of the component characters studied, total tree height and age had the maximum direct effect on diameter. However, height of crown had maximum negative direct effect and indirect effect by self-pruning ability. It also had a strong positive indirect effect via total height and clear bole height.

2.1.3 Heritability and Genetic Advance

2.1.3.1 *Tamarindus indica* L.

Heritability estimate has an important role in tree improvement, as it provides an index of the relative strength of inheritance versus environment. High heritability combined with high genetic advance can be a good indicator and that particular character can be selected.

Genetic estimates of half-sib families of twenty *Tamarindus indica* L. genotypes showed for the characters, phenotypic coefficient of variance PCV was higher than Genotypic coefficient of variance (GCV) indicating the influence of environment Hanamashetti, (1996). Heritability (broad sense) was
medium in respect of all the characters except leaflet length, which was low. Genetic advance as percent over mean was high for germination percent, height and number of leaves and was medium for leaf length, leaflet length and diameter. By genetic analysis it was found that, pulp weight per pod is highly controlled by genetic influence rather than environment alone, hence there is a tremendous scope for tree improvement. For pod traits, shell weight, pod weight, vein weight and pulp weight exhibited high heritability, these traits exhibited higher genetic advance.

Divakara (2002) investigated the genetic control over biometric traits, pod characters, seed parameters, seedling attributes and pulp biochemical traits. The results revealed that among the biometric traits, volume index recorded high heritability (52.2%) and genetic advance (GA) as percentage over mean (27.07%). For pod traits, shell weight (89.14 and 101.27%), pod weight (88.72 and 109.13%), vein weight (87.42 and 152.88%) and pulp weight (87.40 and 141.88%) exhibited higher estimates of heritability coupled with higher genetic advance. Among eleven pulp biochemical traits, anthocyanin pigment registered good heritability (99.6%) and very high GA (83.12%).

2.1.3.2 Other Tree Species

The heritability (broad sense) was found to be the highest for height (0.93) followed by number of branches (0.82), basal diameter (0.76) and wood biomass (0.49) in Gliricidia sepium as reported by Rajaram (1990).
Kumaran (1991) obtained maximum heritability (0.98) for height in the seedlings of *Pongamia pinnata*.

Singh and Beniwal (1993) estimated the heritability in *Bambusa balcooa*. The heritability estimates during different age groups varied between 0.78 to 0.96, 0.82 to 0.99 and 0.40 to 0.89 respectively for height, girth and number of new shoots in *Bambusa balcooa*.

2.1.4 Multivariate Analysis

2.1.4.1 *Tamarindus indica* L.

Multivariate analysis is useful in presenting more convincing evidence of the phenotypic relationships within the plant population under natural and artificial Selection. It is also said to have a capacity of detecting unsuspected relationships between the populations (Sneath and Sokal, 1973). It has been successfully used to classify the biological populations and to identify the factors influencing their genetic divergence (Anderson, 1958; Murthy *et al.*, 1965; Narayan and Macefield, 1976).

The multivariate studies help in isolating the genotypes that are distinctly related and bringing together the closely related genotypes. Earlier studies have shown genetic diversity in space and time (Thoday 1953; Dacunha and Dobzhansky, 1954).
The extent of genetic divergence is crucial for productive heterosis breeding programme. The extent of heterosis has been related to genetic diversity of parents in a number of species (Hagberg, 1952; Sriwatatopongse and Wilsie, 1968; Cange and Carlson, 1973).

The exact distribution and moments of $D^2$ statistic was given by Bose (1936). Rao (1948) pointed out that $D^2$ statistic needed some mathematical and logical requirements such as: distance between the two groups was not less than zero, sum of distances of a group from two other groups is not less than the distance between the two groups similar to that of differential geometry, the distance should not decrease when additional characters were included, and the increase in the distance, by the addition of some characters to suitably chosen set was relatively small.

Rao (1960) opined that $D^2$ concept lies at the heart of the morphometrics, which has lead many useful inferences and thus has become an indispensable tool.

Gangaprasad (1993) studied 282 genotypes of *Tamarindus indica* L. belonging to eight different provenances of six districts, were included to assess the nature of genetic diversity by multivariate analysis. Pod yield per tree is the most important character contributing for divergence across the provenances followed by trunk length, pulp yield per tree and seed yield per
tree. Clusters formed on the canonical graphs were well discriminated indicating the prevalence of appreciable amount of genetic divergence.

Hanamashetti (1996) studied 40 genotypes of *Tamarindus indica* L. belonging to five districts were included to assess the nature of genetic diversity by multivariate analysis. The pulp weight alone contributed maximum in discriminating the genotypes. 40 genotypes were grouped into 10 clusters, Cluster -IV had the highest mean value (3.15 ) for pulp weight.

Divakara (2002) evaluated the genetic diversity of thirtyfive *Tamarindus indica* L. clones and found that, the euclidian distance for the pair wise combination of clones were highest (3.31) between the clone KABANG TNCBPC.

2.1.4.2 Other Tree Species

Khosla *et al.* (1979) reported a multivariate analysis in *Populus ciliata* showing no difference between the male and female trees with respect to height, diameter and specific gravity.

Surendran and Chandrasekaran (1988) found highly divergent clusters in *Ducalyptus tereticornis*. The study revealed that the variability exhibited by the single tree at 24 months growth phase was primarily due to number of branches, number of leaves and plant height, and secondly due to internodal length, leaf length, breadth ratio and leaf length.
Rajaram (1990) studied the genetic divergence among 15 provenances of *Gliricidia sepium* resolved into two clusters at 24 months growth period of the eight characters studied height (58.09%), followed by number of branches (20.95%), contributed maximum to the divergence. The inter and intra cluster distance revealed that Cluster-11 possessed higher values than Cluster-1,

Kumaran (1991) could find two clusters among 28, one parent families in *Azadirachta indica* (neem). Among the characters studied root length followed by height contributed maximum to the divergence. The intra cluster distance of Cluster-1 was 27.46 and that of Cluster –11 was 33.48. Cluster-11 recorded the maximum inter cluster distance of 112.1 followed by 753.88 of Cluster-1. In another study

Kumaran (1991) opined that all the 28 one parent families were grouped into three Cluster after D^2 analysis in *Pongamia pinnata*. The study revealed that height (36.24%) contributed maximum to the divergence followed by number of leaves (22.22%) and leaf length (20.11%). The intra and inter cluster distance values were maximum for Cluster –111.

Bagchi (1992) made a preliminary on the genetic divergence of *Acacia nilotica*. It revealed that all the 42 provenances could be grouped into two clusters based on D^2 values. This study also signified that all seed sources
from a single cluster and the pattern of genetic nearness was not dependent on the geographical nearness.

Singh and Chaudhary (1992) made the multivariate analysis of genetic diversity among 28 single tree selections of *Prunus armeniaca* L. (plus trees) through their progenies for five developmental characters *viz.* Plant height, basal diameter, number of branches, leaf length and leaf breadth at 12 months growth phase. The results revealed that they could be grouped into three clusters. Inter cluster distance (D value) ranged from 5.95 to 6.48. While intra cluster distance was found to be maximum (3.09) in Cluster –1. Plant height and number of branches contributed considerable, accounting for 65 percent of total divergence. The study also revealed that Cluster – 11 and Cluster – 111 were highly divergent and were likely to produce new genotypes with desired traits.

### 2.1.5 Mahalanobis $D^2$, Statistic and Clustering Analysis

#### 2.1.5.1 *Tamarindus indica* L.

For discriminating any two populations having unknown origin, anthropologists were using Carl Pearson's Racial likeliness identity ($C^2$) (Trisdesley, 1921). But Mahalanobis (1936) opined that Pearson's $C^2$ was a test of divergence between two samples than a measure of actual magnitude of divergence between the two groups under comparison.
Mahalanobis generalized, "distance ($D^2$ technique) considers the variations produced by any character and the co-joint effect that it bears on the other characters". The technique first used by Mahalanobis et al., (1949) in an anthropometrical survey of the united province of India.

Samiullah (1993) employed principal component analysis to study the genetic diversity for twenty yields and yield attributing characters across 300 genotypes of *Tamarindus indica* L. The first 12 principal components accounted for 96.63 per cent of variation, while the first two principal components explained 42.37 per cent of variation. Pod yield per tree, trunk length and number of primary branches were the most important characters that contributed towards divergence. Clustering was done based on the principal component scores and all 300 genotypes studied were grouped into 16 clusters.

Gangaprasad (1993) studied the genetic divergence in *Tamarindus indica* L. clusters formed on the canonical graphs were well discriminated indicating the prevalence of appreciable amount of genetic divergence. Genotypes 4 and 10 from Shimoga, 18 and 10 from Chitradurge-1, 4 and 8 from Chitradurga-11, 14 and 34 from Bangalore, 8 and 33 from Tumkur, 13 and 9 from Mandya, 8 and 28 from Kolar-1, and 42 and 25 from Kolar-11 were identified as the most divergent based on canonical variate analysis.
Hanamashetti (1996), in the canonical variate analysis, the number of variables were reduced to a linear expression called principal component and this accounts for most of the variation produced by these characters. A second succeeding principal component was also formed to account for the residual variability. The principal component which is a linear expression of the several variables assigns separate weights to each variable depending upon the ability of each character to discriminate the entries. The first principal component was significant enough to use for the purpose of discriminating the genotypes and to cluster on the canonical configuration. The contribution of the remaining vectors was very low and hence, they were not considered.

2.1.5.2 Other Tree Species

Verma et al., (1973) studied the nature of genetic divergence and its relation to adaptability in seventy-eight varieties of soybean from different countries. The D^2 analysis showed that these varieties formed eleven clusters, which were heterogenous with respect to place of origin of the varieties. They observed a regular clustering pattern with respect to the early and late varieties. The Canonical analysis applied for the same data showed that days to flowering followed by 100-grain weight, days to maturity and plant height contributed more towards discriminating the groups. The congregates formed on canonical graphs supported the clusters formed through D^2 analysis.

Ganeshiah (1979) studied the magnitude of genetic diversity and pattern of association between yield and other characters in a collection of 100
entries of horse gram and grouped them into 23 clusters. He also observed perfect agreement between canonical variate and $D^2$ analysis. The characters that contributed for the divergence are mainly associated with plant maturity. No perfect association was evident between geographic and genetic diversity.

Maluf et al., (1984) assessed the tolerance for aluminum in leucaena and based on hierarchial cluster analysis, distinguished the population into 13 tolerant and 16 sensitive groups.

Melendreas and Ortuno (1984) analyzed the data of several varieties of lemon on various root stocks and were able to group them into different clusters based on Dendrogram, solely depending on fruit characters and arranged them in hierarchial order of similarity.

Nema and Sharma (1986) studied the taxonomic relationships of 42 cultivars using data on 92 physical characters in grape. They were able to classify the cultivars into several groups based on weighed pair group and similarity and distance coefficient method.

Butenko et al., (1987) applied principal component analysis based on 18 quantitative characters in 20 cherry genotypes. Six principal components accounted for 86.2 per cent of the observed variation while the first principal component alone accounted for 35.9 per cent.
Hilling and Fezzoni (1987) analysed divergence in 16 cultivars of Sour Cherry using principal component analysis. They also revealed that genetically related cultivars tended to cluster, suggesting that there was a significant genetic component to the underlying pattern of morphological variation and has also contributed to the patterns. Selective forces may also have contributed to the patterns. Selective forces may also have contributed to the patterns of morphological variation.

Singh et al., (1987) measure the genetic distance of twenty Indian cultivars of Sugarcane using Mahalanobis D^2 statistics. The cultivars were grouped into nine clusters. There was no apparent link between geographic distribution and genetic diversity.

Illoh and Olorode (1990) subjected 31 varieties of mango collected from all the ecological zones to single linkage cluster analysis. The varieties were grouped into four clusters mainly on the basis of reproductive characters.

2.2 GENETIC DIVERSITY

2.2.1 Tamarindus indica L.

Markers based on DNA sequence variation are increasingly being utilized for construction of genetic maps and marker-assisted selection. Application of molecular markers in plant breeding has established the need for information on variation in DNA sequence. DNA-based markers provide a
reliable means of estimating the genetic relationships between genotypes and
taxonomic groups as compared to morphological markers (Gepts, 1993)

Molecular tools such as DNA markers are increasingly becoming important as effective tools in breeding programs. Recently developed DNA-based markers like Restriction Fragment Length Polymorphism (RFLP) and Randomly Amplified Polymorphic DNA (RAPD) provide excellent tools to study the genetic diversity, eliminate duplicates in germplasm, study genetic relationships, gene tagging, genome mapping, PVR (Plant Varietal Rights) purposes etc. These markers measure diversity at DNA level and are seldom influenced by environmental conditions as in case of morphological markers.

In *Tamarindus indica* L. no attempt has been made to evaluate the potential value of genetic markers in characterizing the genotypes and for tree improvement. The present study envisages in assessing the diversity at molecular level and superior genotypes can be identified for documentation.

*Randomly Amplified Polymorphic DNA* (RAPD) markers:- This method of DNA polymorphism analysis was developed independently by two different Biotechnologists (Welsh and Mc Clelland, 1990; Williams *et al.*, 1990). This procedure detects nucleotide sequence polymorphisms in a DNA amplification-based assay using only a single primer of arbitrary nucleotide sequence using Polymerase Chain Reaction (PCR). Each primer will direct the amplification of
several discrete loci in the genome, making the assay an efficient way to screen for nucleotide sequence polymorphism between individuals.

One attempt has been made by Boukary et al. (2007) using RAPD markers for estimation of genetic diversity in *Tamarindus indica* L. to find the clues of the origin of the species. Seeds were collected from Asia (India and Thailand), Africa (Burkina Faso, Senegal, Kenya and Tanzania) and from islands of (Madagascar, Reunion and Guadeloupe). Due to the high value of intra-popular variability, populations did not determine the origin of the species.

2.2.2 Other Species

Simon *et al* (2007) have showed morphological and molecular analysis of genetic diversity in Jackfruit (*Artocarpus heterophyllus* Lamk.) using RAPD markers. 12 high yielding accessions were obtained from different locations of southern India. PCR amplifiable DNA was isolated using the Cetyl Trimethyl Ammonium Bromide (CTAB) method and 171 amplified fragments were obtained using 23 random primers. The genetic dissimilarity based on squared euclidian distance revealed a maximum genetic distance of 7.9% between clones of ‘Mottavarica’ (M0) and ‘Chandrahalasu’, from distant locations. Minimum genetic distance (5%) was between gentoypes (M0) and ‘Kerala’, which are from similar geographical origion. On the basis of geographical location of all genotypes expressed two clusters on dendrogram. The study exhibited low to moderate genetic diversity among 12 jackfruit accessions,
which assists in the identification and management of jackfruit germplasm for
tree improvement.

Cai et al., (2006) worked on the genetic variation in cherry germplasm
using RAPD analysis. Random amplified polymorphic DNA (RAPD) variation
among eight cherry species and two interspecific progenies were analysed.
Out of 130, 48 arbitrary oligonucleotide primers were screened for PCR
amplification to generate polymorphism. A dendrogram was generated to show
relationship among the species and cultivars. The results showed 840
amplified loci. The range of genetic distance was from 0.0623 to 0.2719
among Prunus species. This showed a wide range of genetic variation.

Preliminary RAPD tests were carried out by Lewis et al., (1992) on
avocado cultivars ‘Hass’, ‘Fuerte’ and ‘Edranol’ indicated that three arbitrary
synthetic DNA primer sequences homologous to regions of the avocado
chromosomal DNA. The products of the PCRs were separated by
electrophoresis and the presence or absence of bands statistically analysed to
determine similarities between the cultivars. ‘Fuerte’ and ‘Edranol’ were found
to be closely related.

The genetic relatedness among 17 almond genotypes and one peach
genotype was estimated using 37 RAPD markers. Genetic diversity within
almond was found to be limited despite its need for obligate out crossing.
Three groupings of cultivars origins could be distinguished by RAPD analysis.
A similarity index based on the proportion of shared fragments showed relatively high levels of 0.75 or greater within the almond germplasm. The level of similarity between almond and the peach was 0.424 supporting the value of peach germplasm to future almond genetic improvement (Bartolozzi et al., 1998).

RAPD analysis of *Olea europaea* L. (olive) cultivars was carried out by Fabbri et al., (1995) reported a high degree of polymorphism in the germplasm examined. Forty random decamer primers were screened. Seventeen of these produced 47 reproducible amplification fragments useful as polymorphic marker. Each of the 17 cultivars was discriminated with a few primers. Results were analyzed for similarity among the cultivars and a cluster analysis was performed. This analysis revealed two main groups: one comprising primarily small-fruited cultivars grown mainly for oil production and the other characterized by having large fruit. There was no apparent clustering of Olive cultivars according to their geographic origins.

Molecular characterization of common olive varieties in Israel and the West Bank using RAPD markers carried out by Wiesman et al., in 1998. Significant biodiversity was demonstrated in 'Nabali' olive tree grown along the central mountain ridge of West Bank, suggesting that the grouping known as 'Nabali' is actually a mixture of genetically distinct variants. On the other hand, RAPD profiles of selected variants of 'Souri', cultivated mainly in the northern mountains of Israel, revealed a high degree of similarity, indicating that these
variants represent environmental phenotypes of the same genome. Molecular
differences were demonstrated between the 'Nabali' group variants and 'Souri'.
Other more recently developed or introduced varieties showed individually
distinct RAPD profiles.

Feng et al. (1997) studied relationships among species in Morus L.
using RAPD markers. Relationships among operational taxonomic units (12
species and 2 varieties) of Morus were examined with 20 random primers,
generating 238 polymorphic RAPD bands. According to dendrogram
constructed using Nei's genetic similarity values and an Unweighted Pair
Group Method with Arithmetic mean (UPGMA) program, M. notabilis was far
removed from the other species, indicating its unique differentiation. All
groups were identical with morphological classifications, indicating the
usefulness of RAPD for systematic studies.

Detection of genetic diversity was done using RAPD-PCR for sugar
analysis in (Citrullus lanatus (Thunb.) Mansf.) watermelon germplasm (Lee et
al., 1996). RAPD markers generated by 15 arbitrary decamers were used to
determine the frequency of DNA polymorphism in 39 watermelon germplasms.
Of the 15 primers tested, all except one (primer 275) directed the amplification
of polymorphic products. A total of 162 fragments, 35 (21%) appeared to be
reliable polymorphic markers. The mean value by marker difference in this
comparison was 0.24, and the highest, 0.69. From the phenograms
constructed by UPGMA based on the comparison of RAPD markers, four
clusters were resolved. Each group was also characterized and identified with morphological and genetic characteristics for each genotype. Results from the phylogenetic analysis of band sharing data were consistent with sweetness as measured by High Performance Liquid Chromatography (HPLC). In conclusion, RAPD assays can be used for providing alternative markers for identifying genotypes and quantitative characteristics (such as sweetness) in watermelon.

Samec and Nasinec (1996) reported the use of RAPD technique for the identification and classification of *Pisum sativum* L. genotypes. The genomic DNA s of 42 *Pisum sativum* genotypes, representing four wild and cultivated subspecies were used as templates in RAPD reactions. Amplification with eight decamer primers generated 149 polymorphic products. Genetic similarities of RAPD profiles were estimated via a coefficient of Jaccard and then the data were processed by cluster analysis (UPGMA). Each genotype was clearly identified and separated from the others. These results show that RAPD technology is a rapid, precise and sensitive technique for identification of pea genotypes.

### 2.3 FOLIAR VENATION IN CLASSIFICATION OF TAXON

The leaf venation pattern is considered as taxonomic evidence to classify the species. According to Hickey (1973, 1974), leaves are the neglected organs in taxonomic and morphological studies due to lack of unified, unambiguous and detailed classification of their features. Dilcher
(1974), says that large number of angiospermous plant fossils, represented by leaf impressions have not been identified due to lack of basic information on the epidermis, venation and cuticular characters. Ettingshausen (1861), Monton (1970) and Sehgal and Paliwal (1974) have contributed to the enrichment of the knowledge on leaf venation. Now there is need to stress for the extensive and intensive studies on venation pattern in the leaves of angiosperms.

The works of Hickey (1973, 1974), Seetharam and Kotresha (1998), which helps us to view the architecture of dicotyledonous leaves, particularly the shape of the leaf base, apices, marginal teeth and the venation pattern.

An attempt has been made to study the venation of *Tamarindus indica* L. and use the information to differentiate the genotypes. The terms used to describe the venation pattern are drawn from the works of Melville (1976), Pascal & Ramesh (1987) and Radford et al., (1974).

### 2.4 BIOCHEMICAL ANALYSIS

*Tamarindus indica* L. fruit pulp is an important ingredient for souring curries, sauces and certain beverages. The pulp contained proteins, reducing sugars, phenols, pectin and tartaric acid besides fiber and cellulose material, Lewis et al., (1954). According to him acid and sugar content varied from sample to sample.
2.4.1 Reducing Sugars in *Tamarindus indica* L.

*Tamarindus indica* L. fruit contains reducing sugars ranged from 25-45%. Compared to tender fruit pulp, riped fruit contains more sugar. By ripening the acid level is decreased, sugar level increases to 30-40%, giving the sour fruit a sweeter taste. (Lewis and Neelakantan, 1964; Coronel, 1991). *Tamarindus indica* L. fruit contained 41.20 per cent of total sugars, 25.00 – 45.00 per cent of reducing sugars and 16.52 per cent of non-reducing sugars (Shankaracharya, 1998).

2.4.2 Phenol Diversity in *Tamarindus indica* L.

Phenols in fruit pulp provide resistance to plant. Apart from this phenols also act as antioxidants in human diet. Phenols include a large group of organic aromatic compounds having one or more hydroxyl groups on the benzene ring. They are known to provide resistance to plant. However in recent interest in food phenolics has increased, because of their antioxidant and free radical scavenging abilities and their implication in the prevention of disorder such as cancer and cardiovascular disease. Epidemiological studies have shown a correlation between an increased consumption of phenolic antioxidants, reduced the risk of cardiovascular disease and certain types of cancer (Hertog *et al.*, 1993, 1995; Hertog 1996; Rimm *et al.*, 1996; Hollman and Katan 1999). Phenols constitute one of the most numerous and widely distributed substance in the plant kingdom, with more than 8000 phenolic structures currently known (Harome 1993).