Review of Literature
The recent estimates of world-wide reserves of petroleum and natural gas are estimated to be around 1.33 trillion barrels and 6,186 trillion cubic feet, respectively. The world consumes a total of 85.4 million barrels per day of oil and 261 billion cubic feet per day of natural gas. The International Energy Agency (IEA) and the US Energy Information Administration (EIA) Annual Energy Outlook have forecasted a rising trend for oil prices from US$100 to 160 per barrel by 2050 (Anon., 2011). At the rate of current consumption, the world-wide oil reserves will be exhausted by 40 years, and reserves of natural gas by 60 years (Vasudevan and Fu, 2010). Among the renewable and non-renewable energy sources, the latter energy source predominantly includes fossil fuel such as oil and coal, which do not provide sustainable and environmental friendly benefits. The continuous burning of fossil fuel has not only led to the diminishing of petroleum reserves, but also to an urgent need to develop alternative, renewable and environmental friendly fuel. The renewable energy sources include solar, wind and geothermal energies and biomass and hydropower. Biofuels, produced from selected agricultural biomass, among other renewable sources, provide for sustainable and eco-friendly energy options.

The sources of biofuel are vegetable oils that are usually extracted from oilseeds, nuts, fruits, and waste cooking oil or animal fats. Biofuels derived from biomass might occur in the form of solid, liquid or gas. The solid fuels like wood, agricultural wastes and biomass briquettes are used commonly in rural areas. The liquid fuels are bioethanol and biodiesel produced from sugarcane, molasses, corn, sugarbeet, lignocellulosic,
cellulosic materials, oilseeds and animal fat. The gaseous fuel include biogas produced from anaerobic digestion of animal waste, house-hold waste and other municipal wastes and producer gas from the pyrolysis of agricultural wastes and wood (Malenshek and Olsen, 2009).

**Biofuel – an emerging renewable option**

The usage of biofuel will help in balancing the agriculture and economic sector and prevent the environmental pollution. Chemically the oils/fats consist of triglyceride molecules of three long chain fatty acids that are ester bonded to a single glycerol molecule. These fatty acids differ by the length of carbon chains, the number, orientation and position of double bonds in these chains. The biodiesel is lower alkyl esters of long chain fatty acids, that are synthesized either by transesterification with lower alcohols or by esterification of fatty acids. The importance of taking up a major program on the use of biofuel for blending with gasoline and diesel in our country is justified by a variety of factors. First, there is no alternative to the petroleum-based fuels in the transport sector which is one of the major consumers. Secondly, biofuel is environmentally superior fuels and their use becomes compelling if the prescribed emission norms are to be achieved. Thirdly, there is need to meet the global environmental concern about climate change, ensure energy security, reduce imports and generate employment for the poor, and achieve a number of other objectives like benefits to rural community in growing biofuel plants (Dufey, 2006).

**International status of biofuel program**

More than 20 countries have already initiated the use of biofuel since 95% of the global oil is consumed for transportation alone and oil prices have increased in the
international market. About 100 biofuel bioreactor plants are operational in USA, Austria, France, Italy, Hungary, Germany, Czechoslovakia and UK. There are many countries that are involved in the bioethanol and biodiesel production. Tax support is being provided for biodiesel promotion. In the European Union, the dominant biofuel is biodiesel (EU member states produce about 89% of the world's biodiesel. Fig. 1), while ethanol is the dominant biofuel in the United States. The biodiesel provides for about one per cent of all transport fuels in Europe, while ethanol provides two to three per cent of light-vehicle transport fuel in the United States (Anon., 2008; Anon., 2012).

**Fig. 1. Biodiesel production in European Union (2000-2011)**

![Biodiesel production graph](image)

*Source: Anon. (2008), Anon. (2012)*

**Status of biofuel program in India**

India is fifth in the world in energy demand accounting for 3.5% of world commercial energy consumption. India is one of the largest importers of edible oil. India has approximately 5,52,692.25 sq. km of wasteland (Anon., 2010a), which could be utilized for cultivating biofuel crops. Indian sub-continent with its rich biodiversity has more than 400 species of plants yielding oil which could be commercially exploited for
the production of energy. A number of ventures and policies are being devised by both central and state governments for increased production and commercialization of biofuel in India. It is expected that the demand for biofuels from vegetable oils and fats will shoot up to 3 million tons a day.

The energy plantations on wasteland serve as a micro-enterprise for land-less pro-poor community at rural sector (Kesari and Latha, 2010; Savita et al., 2010). A number of major organizations have taken up the biofuel segment including National Oil Seeds Vegetable Oils Development Board (NOVODB) (Anon., 2010b), Petroleum conservation Research Association (PCRA), The Energy and Research Institute (TERI) (Bhojvaid, 2008), The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Anon., 2007b) and Indian railways. Objectives of these organizations are to rehabilitate the degraded land, protect environment, establish network program for identifying elite planting material and consolidate plans for cultivation of non-edible oil trees. In the international and national perspective, Ministry of New and Renewable Energy (MNRE) has formulated the National Biofuel Policy in 2008 aiming at centralizing the biofuel program in energy and transport sector in the country with the support from states and to facilitate the development and utilization of biomass feedstock for biofuel production (Anon., 2008b).

**Status of biofuel program in Karnataka**

The degradation of land due to improper utilization and increase in population has resulted in the generation of large stretch of wasteland in Karnataka. As per the statistical estimate, the wasteland in Karnataka is 20,839.28 sq. km (Anon., 2010a), which is unfit
for the cultivation of agriculture crops but can be utilized for biofuel plantation. The key organizations involved in biofuel plantations in Karnataka are the Department of Agriculture, University of Agricultural Science (UAS), South Western Railways, Karnataka State Road Transportation Corporation (KSRTC), Bangalore Metropolitan Transport Corporation (BMTC) and Karnataka Forest Department (KFD). These organizations have taken up initiatives to successfully utilize the alternative source of energy-biofuel in Karnataka. The trial run of BMTC buses by the use of alternative eco-friendly fuel in place of diesel contributed to national oil conservation. The objectives were to check the mileage improvement in comparison to diesel under regular road conditions, to reduce the vehicular pollution and improve the image of BMTC as a “Green Transport System” using biofuel. The experimentation resulted in significant increase in the mileage.

In 2006, University of Agriculture Science, Bangalore established the ‘Biofuel Park’ in Madenur, Hassan as a “mega model”. The Biofuel Park was established to generate the systematic replica of model nursery, production unit, marketing system and other biofuel programs in other parts of the country (Gowda et al., 2009). Karnataka is the first, among the other states of India, to have declared the State Biofuel Policy in the year 2009 under the ministry of Rural Development and Panchayat Raj (RDPR) (Anon., 2009; Sarvesh et al., 2008). Karnataka State Biofuel Development Board has been set up in the year 2010 with the objective to link various aspects of biofuel program. The strategies suggested for the full-scale launch of biofuel activities include combining of various schemes under a single umbrella and its implementation, adequate focus to all type of biofuel crops, and the creation of awareness among farmers, identifying
non-governmental organizations (NGO) as a link between farmers and financial institutions, engaging students and formation of an exclusive society of farmers, similar to the milk federation.

**Sources of biodiesel**

Sources of biodiesel are generally dependent on the prevailing climatic conditions in a particular region. Currently, more than 95% of the world biodiesel is produced from edible oils which are easily available on a large scale from the agricultural industry. The edible oilseed crops such as soybean (*Glycine max* L.), canola or rapeseed (*Brassica* spp.), groundnut (*Arachis hypogaea* L.), safflower (*Carthamus tinctorius* L.) and linseed (*Linum usitatissimum* L.) are being used for biodiesel production. In Brazil, castor oil (*Ricinus communis* L.) was initially promoted for more than a century for lubrication and plastics and now it is being introduced as a source for biodiesel production (Gressel, 2008). In Europe and Canada, rapeseed oil methyl ester (RME) produced from oilseed rape is the main substitute fuel. The US ranks first in soybean production (8.24 million tonnes), followed by Brazil, Argentina and China. Oil palm has been utilized for commercial purposes in Indonesia and Malaysia (Anon., 2012a).

**Edible oilseeds**

*Brassica* species

The oil of plant origin finds a lot of importance in human consumption and industries. In order to meet demands of every day requirement, many plant species have been domesticated and cultivated for centuries. *Brassica* species of Brassicaceae is one
among the plant species cultivated extensively for oil both for the edible and industrial purposes including biodiesel production throughout the world (Dworakowska et al., 2011). The *B. napus* L. and *B. rapa* L. commonly called as oilseed rape or industrial rapeseed and canola with low erucic acid, a hybrid of *B. napus* and *B. rapa* are the preferred species for biofuel production. The direct effect of high altitude on seed yield was reported in one of the genera (Dierig et al., 2006). *Brassica* species generally contain high levels of erucic acid which is undesirable for human consumption (Snowdon et al., 2007).

The association studies in *Brassica* species forms the prerequisite information in plant breeding program for high oil content with desired fatty acid. Singh et al. (2002) studied the association of fatty acids like oleic acid, linoleic acid and linolenic acid in rapeseed. Acharya (2006) observed a positive and direct effect of seed yield and weight on oil content in 58 F 2S populations of *B. juncea*. Khan (2008) reported that the phenotypic co-efficient of variation was higher than the genotypic co-efficient of variation in the brassica genotypes from Peshwar. Yousuf et al. (2011) showed that rapeseed accessions with greater similarity for agro-morphological and seed quality traits were placed in the same cluster. Sutariya et al. (2011) showed that the geographical location played a major role in grouping of the Indian mustard genotypes. The sodium dodecyl sulphate poly-acrylamide gel electrophoresis (SDS-PAGE) was used for studying the genetic diversity in *Brassica* species and inter-specific genotypes of brassica cultivars (Munazza et al., 2009; Rahman et al., 2004). The AFLP markers were studied to assess the genetic diversity among 21 natural and synthetic varieties and lines of *B. juncea* from different countries (Srivastava, 2001) and such markers could be further
used for the development of SCAR markers tagged to specific agronomic traits (Negi et al., 2000).

The seed development in many of the Brassica species has been reported and the decrease in breakdown of chlorophyll content during low temperatures during the ripening of seeds in B. napus was reported by Ward et al. (1992). Kang and Rawsthorne (1994) reported that in brassica during both fatty acid and starch synthesis, glucose-6-phosphate is one of the precursors in the initial step of seed biosynthetic pathway in plastids. Wiberg et al. (1997) monitored the fatty acid composition along with storage lipids and cell membrane lipid content during the seed development in two transgenic B. napus. Temperature is known to play a major role in the accumulation of lipid in B. napus (Deng et al., 1998). The electrical conductivity was found to decrease during the seed development in B. oleracea (Guruswamy and Thiagarajan, 1998). Katavic et al. (2006) reported that the protein subunit band between 20 and 19 kDa corresponded to oleosins in B. napus cultivars. The gradual decrease in soluble sugar during seed development has been reported in rapeseed (Morley-Smith et al., 2008). The ultrastructural studies on the seed reserve materials have been reported in brassica. The correlation between high oil content and small oil bodies using transmission electron microscope was reported in B. napus (Hu et al., 2009).

**Glycine max**

Glycine max (L.) Merr. (soybean) of Fabaceae is the dominant oilseed crop produced in the world, due to certain valuable characteristics like high-quality protein and edible oil. It contributes over a half of all oilseeds produced world-wide. The US
ranks first in soybean production (8.24 million tonnes), followed by Brazil, Argentina, China and EU-15 (4.28, 3.28, 3.26 and 2.87 million tones, respectively Anon., 2012a.

The soybean oil consists primarily of neutral lipids (tri-, di- and monoacylglycerols), free fatty acids, and polar lipids such as phospholipids with minor amount of unsaponifiable matter that includes phytosterols, tocopherols, and hydrocarbons such as phospholipids. In recent years, soybean oil has been used for biodiesel and ethanol production (Koc et al., 2011). Kumar et al. (2006b) reported that the latitude has no correlation with the fatty acid content of seven Indian cultivars. The genetic diversity studies in soybean using seed protein profiling showed no relationship between the geographical origin and clustering pattern (Faisal et al., 2009).

Many studies have been conducted on seed development in soybean. Blackman et al. (1992) observed that monosaccharides were high during the initial stages of soybean seed development, while oligosaccharides are high at the later stages of development, promoting desiccation tolerance. A sharp decrease in iron and chlorophyll contents was also reported (Laszlo, 1991) while potassium content remained constant during the seed development (Laszlo, 1994). The electrical conductivity of developing soybean seeds was shown to change depending on the genotype (Panobianco and Vieira, 1996). The unsaturated fatty acids are required for lipid membrane biosynthesis in soybean which enhances the tolerance of seed to cold temperature (Lanna, 2005). A steady increase in oil content on dry weight basis during the development of soybean seed was reported by Ghassemi-Golezani (2010). Sharma et al. (2011) reported a gradual decrease in soluble sugar and total lipid content during the soybean seed development. Studies on seed composition with regard to decrease in content of amino acids like Met and Cys at high temperature have been reported (Carrera et al., 2011).
Arachis hypogaea

*Arachis hypogaea* L. (groundnut), another species of Fabaceae, is the world’s fourth most important edible oilseed with high protein content, vitamins and thiamine. The major producers of groundnut are India, China, USA, Indonesia and Australia accounting for two thirds of world production. India accounts for 6.6 m ha of global area and contributes to 5.9 million tons of groundnut production (Anon., 2007). Ahmad et al. (2009) reported that groundnut oil could be used for the production of biodiesel. The natural variability in groundnut is negligible, accounting for its low out-crossing nature. Much emphasis has been given for the morphological descriptors and selection based on these descriptors and maintenance of elite germplasm. Upadhyaya (2003) reported 16 morphological descriptors and 32 agronomic traits in a core collection of 1,704 accessions of two sub-species. The two sub-species differed significantly in pod length and width, seed length and width, yield per plant and 100 seed weight with significant phenotypic correlation. Groundnut is the most important source of protein and feed in developing countries. The nutritional qualities like oil content, crude protein, total carbohydrates and mineral nutrient contents of 20 groundnut varieties grown in Ghana and significant genetic variability were reported by Asibuo et al. (2008). Wang et al. (2011) reported 36.38 to 80.51% of oleic acid and 2.91 to 41.82% of linoleic acid content in 152 groundnut genotypes, including 38 released cultivars, 34 landraces, 15 breeding lines and 36 inter-specific derivatives. Germplasm with high protein, oil content, sucrose content and oleic acid to linoleic acid ratio were identified for breeding studies.

The genetic variations using molecular markers were identified for breeding programs in groundnut. Javaid et al. (2004) reported low genetic diversity in 151
groundnut landraces from five continents for total seed storage proteins by SDS-PAGE. Dwivedi et al. (2001) reported the genetic diversity in 26 accessions of groundnut consisting inter-specific, land races and released cultivars using eight RAPD primers. The genetic similarity between accessions ranged from 59.5 to 98.9%. More recently, the most reproducible and reliable molecular markers have been employed in studying the genetic diversity in groundnut germplasm. The AFLP markers were used to study the genetic diversity among 100 groundnut landraces from the Central, Western, Southern and Lake Victoria agro-ecological regions of Tanzania (Ntundu et al., 2004). Eleven informative AFLP primer combinations generated 49 polymorphic fragments across all accessions and the effect of geographic isolation on clustering was reported. Genetic variability and correlation studies across 34 genotypes for different yield parameters with significant variations were reported by Zaman et al. (2011). The seed yield showed high correlation with nut size, number of nuts per plant, kernel size and days to 50% flowering across all accessions.

Carthamus tinctorius

Carthamus tinctorius L. (safflower) of Asteraceae, a multipurpose crop, grown for centuries in India for the orange-red dye (carthamin) extracted from its brilliantly colored flowers and for its quality oil rich in polyunsaturated fatty acids (linoleic acid 78%). Safflower is an oilseed crop which is mainly grown in semi-arid regions. It is used as vegetable and industrial oil, for spice processing and birdfeed. Recently, there is a growing interest in production of biofuels from safflower (Khanahmadzadeh and Khanahmadzadeh, 2012). Many investigators have identified the morphometric traits of safflower cultivars. Camas et al. (2007) showed variation in the morphometric traits of safflower cultivars.
content and fatty acid composition in seeds grown in different regions of northern Turkey, with linoleic and oleic acids being the main oil components for all cultivars, and fatty acid composition varied greatly among locations and cultivars. Oil content and fatty acid composition were also examined in safflower varieties planted in winter and spring seasons. A negative correlation was recorded between the palmitic and the stearic acids and also, an inverse relationship between the oleic and linoleic acids in all varieties was established (Cosge et al., 2007). There was also no correlation between geographical origins, morphological traits and molecular markers of safflower genotypes from Iran, based on the clustering patterns (Amini et al., 2008; Golkar et al., 2011).

In the maturing seeds, diacylglycerol acyltransferase activity increased to a maximum during rapid accumulation of lipids and declined with seed maturity (Weselake et al., 1993). Rahamatalla et al. (2001) also recorded very slow triglyceride synthesis during the early stages of seed development in safflower. The decrease in moisture content and palmitic acid and an increase in oil content at the later stages of seed development was also reported by Gecgel et al. (2007).

**Linum usitatissimum**

*Linum usitatissimum* L. of Linaceae is commonly known as flaxseed or linseed. It is a multipurpose herbaceous species cultivated mainly for fiber and oil. Linseed oil is mainly used in the production of soaps, paints and polymers. It is native to regions extending from eastern Mediterranean to Indian sub-continent. In recent years, it is being used for biodiesel production, as a sustainable substitute for fossil fuel (Nabi and Hoque, 2008). The genetic association between seed yield, yield related traits and oil content of
81 geographically diverse Ethiopian accessions of linseed was studied by Tadesse et al. (2009). Further, the seed weight showed negative and indirect effect on the oil content of linseed genotypes. Bayrak et al. (2010) assessed the level of variation in oil content and fatty acid composition across the 81 linseed genotypes of nine countries cultivated in Turkey. The oil content ranged from 23 to 40% and the fatty acid composition of linseed was dominated by C18 fatty acids with 48 to 57% linolenic acid and 11 to 16% linoleic acid. Kiralan et al. (2010) reported the comparative account of fatty acid and mineral content in 23 linseed lines from four European regions. Linolenic acid was found to be predominant in all genotypes that ranged from 49 to 56% followed by oleic acid (19 to 28%) with negative correlation between the two. A significant variability was reported for all the 10 mineral nutrients. Sivaraj et al. (2012) reported variability in 84 accessions of linseed collected from different regions of Andhra Pradesh and Maharashtra for seed traits, oil content and fatty acid composition. DIVA-GIS diversity analysis for omega-3 profile and seed weight suggested promising regions of linseed collection for purposes of germplasm collection, maintenance and breeding.

In order to identify elite genotypes for germplasm management and breeding, a variety of molecular markers have been used. The total seed protein content of linseed was qualitatively analyzed using an array of electrophoretic methods. Major bands were observed from 40 to 55 kDa and small sub-units at 20 kDa, and mapping gels showed heterogeneous banding pattern. The albumin and globulin contents were high in seed powder (Sammour, 1999). Adugna et al. (2006) reported genetic diversity using the morphological and AFLP markers for 60 accessions of linseed from Ethiopia. A wide range of diversity was observed with different clustering pattern for both morphological
and AFLP markers among accessions. Bhat et al. (2011) studied the genetic diversity, using morphological traits and RAPD analysis, among five genotypes of linseed. The variability was observed for the studied morphological traits, and four RAPD primers generated 80 polymorphic bands across all genotypes.

Non-edible oilseeds

The large scale production and utilization of biodiesel from edible oils raises concern since they compete with food, leading to food versus fuel situation. Further, given the large gap between the demand and supply of edible oils, many developing countries can ill afford to use edible oils for biodiesel purpose. In recent years, the biodiesel production from various non-edible oilseed sources has been extensively surveyed. With ever increasing prices of edible oil in the recent past, the used waste vegetable oils and non-edible oils are preferred as potential low priced feed stocks. Various non-edible oilseed plant species bearing high oil content, such as neem (Azadirachta indica), jatropha (Jatropha curcas), pongamia (Pongamia pinnata), hippe (Madhuca longifolia (Koenig) J.F. Macb) and surahonne (Calophyllum inophyllum L.) are being promoted, particularly in India (Azam et al., 2005). The above species could be promoted on waste-lands, bunds of farmland and are generally not cultivated in land meant for food crops.

Azadirachta indica

Azadirachta indica (A.) Juss (neem) of Meliaceae is one of the oilseed tree species that is used as an alternative fuel in diesel engines, in the recent past (Rao et al., 2008). It could be grown in tropical and sub-tropical regions with semi-arid to humid
climates and shows variability within its natural and introduced habitat. The variability across different agro-ecological zones was observed in fruit and seed characteristics, seedling and tree growth characteristics and its genetic diversity. The seed oil content ranged from 28 to 32%. The environmental factors like rainfall, humidity, temperature and altitude affects the growth of A. indica and production of secondary metabolite like azadirachtin. Several studies on effects of environment on the genetic variation in A. indica have been reported. Kumaran et al. (1993) reported significant differences between populations of A. indica from Tamil Nadu, India. They also reported that seed length, oil content and weight of 100 seed as the selection criteria by virtue of its high genotypic coefficient of variance, genetic advance and heritability. However, variation in tree morphology and oil content of A. indica seeds from different agro-ecological zones have been reported by Kaura et al. (1998). High variability was observed for fruit and seed traits like weight, length, yield, azadirachtin and oil content within and between provenances of A. indica (Jindal et al., 1999; Kundu et al., 1998). Rathore et al. (1998) found highly significant variation in oil and protein content of A. indica seeds from different countries like Laos, Nepal, Ghana, Bangladesh, Myanmar and three areas in India. Kundu and Tigerstedt (1998) observed provenance variation for net photosynthesis, stomata conductance and density and seedling characters which positively correlated with the mean annual rainfall. The variability in seed oil content and fatty acid composition has been extensively studied in A. indica by Kaushik and Vir (2000). The oleic acid ranged from 25 to 58% and linoleic acid from 6 to 17% and hence the fatty acid characteristics could be used in the selection of trees for genetic improvement programs. Munoz-Valenzuela (2007) reported that the age of the tree had no correlation with the oil yield in A. indica collected from Yaqui valley in Mexico.
Neem is also used in traditional medicine in India. Neem contains complex group of compounds called limonoids like azadirachtin, meliantriol, salannin and nimbin (Mordue and Nisbet, 2000). Many investigators have reported the variability in azadirachtin content. The azadirachtin content of seeds showed significant variation in genotypes from different agro-ecological zones of India (Rengasamy et al., 1993). The secretory cells in cotyledons are shown to secret secondary metabolites in *A. indica* (Dayanandan et al., 1993). The azadirachtin content was reported to increase with red and black soils and at altitudes above 500 m and a positive correlation between azadirachtin content, saponification value and acid values of oil were established. The neem trees grown in coastal, arid or semi-arid regions had high azadirachtin content while, the sub-humid regions had low contents (Rengasamy et al., 1995). The azadirachtin and oil content was determined in *A. indica* seeds collected from 12 different locations of Tamil Nadu and negative correlation was reported between azadirachtin content and the total number of rainy days during the fruiting season (April to August) (Sridharan et al., 1998).

Singh et al. (1999) used AFLP markers to differentiate Thailand genotypes from the Indian based on clustering pattern with specific accession and agro-ecological zone specific markers. Kaushik et al. (2007c) also reported clustering in *A. indica* from different agro-climatic zones of Haryana. The single primer amplification reaction (SPAR) combined with simple sequence repeat (SSR), mini-satellite sequences and arbitrary sequence primers were employed to assess the genetic diversity among neem genotypes of 15 provenances, showing the existence of greater similarity between provenances (Ranade and Farooqui, 2002).
Rengasamy and Parmar (1994) reported that azadirachtin and oil content varied with seed maturity. At early stages of fruit development, the azadirachtin content was not detected, while at 50 and 60 days after anthesis 0.051% and 0.112%, respectively were recorded. However, Johnson et al. (1996) reported that the concentration of major limonoids varied only slightly with maturity and azadirachtin content was high in green ripening fruits.

\textit{Jatropha curcas}

\textit{Jatropha curcas} L. (physic nut) of family Euphorbiaceae is a drought-resistant perennial plant capable of growing in a wide range of soil and climatic conditions. Studies on the morphometric traits of the jatropha grown in a common region and different agro-climatic regions showed significant results. The seed oil content ranged from 30 to 35%. The morphometric traits were considered important in predicting their contribution to the final yield of the plant along with their heritability (Ratree, 2004). The altitude showed no effect on the oil content of accessions collected from Himachal Pradesh (Pant, 2006). Sunil et al. (2008) reported that it is important to study the specific plant traits in jatropha to predict the promising accessions for the purpose of germplasm conservation and maintenance. Mishra (2009) reported that medium height and many branches could be important desirable character of oilseed yielding plant. The DIVA-GIS application has been used to predict the diversity and distribution of \textit{Jatropha} species in south east coastal zone of India (Sunil et al., 2009b). Mohapatra and Panda (2010) studied variations in different morphological traits in jatropha accessions collected from Bhuvaneshwar. Kaushik et al. (2007a) reported variability in seed traits and oil content in 24 accessions of \textit{J. curcas} collected from different zones of Haryana, India with significant differences in seed size, 100 seed weight and oil content, however the
phenotypic co-efficient of variation was higher than the genotypic co-efficient of variation indicating the influence of environment. Similar studies were conducted by Rao et al. (2008) who selected 32 high yielding CPT from different locations in Andhra Pradesh, for evaluating the genetic association of seed characters, growth and yield. Gosh and Singh (2011) showed variation in seed and seedling characters of jatropha collected from six agro-ecological zones within India and four to six provenances within each zone.

Martinez-Herrera et al. (2006) evaluated the jatropha accessions from four different agro-ecological regions of Mexico and showed that kernels were rich in crude protein (31-35%) and crude lipid (55-58%). The variability in seed oil content and fatty acid composition has been extensively studied in jatropha in Malaysia, Indonesia and Thailand (Emil et al., 2010). Both reports indicated that oleic (42.4-48.8%) and linoleic acids (28.8-34.6%) were the major fatty acids present in the jatropha seed oil; minor saturated fatty acids include palmitic (13.25-14.5%) and stearic acids (7-7.7%).

A number of DNA based markers have been used to study the genetic relationship between different species of Jatropha and genetic diversity among the jatropha genotypes across the world. The non-toxic genotype from Mexico and toxic germplasm of jatropha from India were evaluated for genetic relatedness using RAPD and ISSR markers, and SCAR markers were identified to distinguish these two types (Basha and Sujatha. 2007). Ganesh-Ram et al. (2008) reported no relationship between the species of jatropha by comparing the genetic relationship of eight species using the RAPD markers. Sudheer-Pamidiamarri et al. (2009) used RAPD and AFLP markers to indicate the close similarity between J. curcas and J. integerrima.
Many investigators have reported the robustness of AFLP markers using marker attributes like polymorphism information content, marker index and resolving power. Basha et al. (2009) studied the seed kernel proteins, oil content, ash content and phorbolesters in 72 jatropha accessions from 13 countries, with significant variation in phorbolester content. The Mexican genotypes contained very low levels of phorbolester. The RAPD and ISSR markers could identify high and low phorbolester content among the Mexican accessions. Tatikonda et al. (2009) used AFLP markers to assess the genetic diversity among accessions from six states of India. Shen et al. (2010) also reported the use of marker attributes of AFLP while studying the genetic diversity of jatropha in Hainan and China. The population genetics have been studied in jatropha to determine the distribution variation among regions, populations or within populations. Wen et al. (2010) studied jatropha populations in Indonesia, China and South America using EST-SSR and genomic-SSR markers which resulted in high total gene diversity than the gene diversity within groups indicating that most diversity is not occurring within population.

The seed development in jatropha is important in studying the association between the physical, biochemical and molecular aspects. Annarao et al. (2008) studied the seed development in jatropha with respect to reproductive biology, oil content, lipid profile and sterol content at an interval of five days from one week after fertilization, until maturity by NMR spectroscopy. The moisture content decreased while the seed size increased from stage I to VI and shrunk at later stages. The lipid synthesis was noticed for nearly three weeks after fertilization. The biotic and abiotic stress have significant effects on triacylglycerol synthesis leading to higher free fatty acid concentration during seed development. Xu et al. (2011) reported the temporal expression profiles of 21 lipid
genes involved in different steps of fatty acids and triacylglycerol synthesis in developing seeds of jatropha using qualitative real-time PCR technology. These reports help in understanding the fatty acid biosynthetic pathway and in improving the biosynthesis of triacylglycerol and reduce free fatty acid content in mature seeds.

**Madhuca longifolia**

*Madhuca longifolia* (Koenig) J.F. Macb of family Sapotaceae has been identified recently for biofuel production. The species is found mainly in sub-tropical regions. The seed oil content ranged from 35 to 42%. Although the seed oil is non-edible, it is used as cooking oil by tribal community. Oleic, stearic, palmitic and linoleic acids were the major fatty acids in mahua fat and its lipid classes (Ramadan *et al.*, 2006). Abraham *et al.* (2010) reported the occurrence of enormous variability in seed length, thickness and 100 seed weight in accessions from Tamil Nadu and the Union Territory of Puducherry. The kernel oil content ranged from 45 to 60%. The variability in seed traits and fatty acid composition of 37 accessions of mahua collected from different parts of Tamil Nadu have been extensively studied (Yadav *et al.*, 2011). High heritability levels were also observed for palmitic acid in mahua genotypes. Accessions were clustered into the respective groups based on their fatty acid composition. The oleic (32.9-48.7%) and linoleic acids (9.4-15.4%) are the major fatty acids present in mahua oil and the minor saturated fatty acids include palmitic (11.7-25.9%) and stearic acid (19.1-32.2%).

**Calophyllum inophyllum**

*Calophyllum inophyllum* L. (surahonne) of family Clusiaceae is a medium to large sized tree native to India, South East Asia and Australia. The seed oil content ranged
from 45 to 50%. The oil is used for medicine and in recent times it has been used as one of the feedstock for biodiesel production (Sathyaseivabala et al., 2010). Crane et al. (2005) reported that oleic acid (39.1-50%) is the dominating fatty acid followed by linoleic acid (21.7-31.1%) as the second major fatty acid. The stearic (13.4-14.3%) and palmitic (11-13.7%) acids are the major saturated fatty acids in C. inophyllum and C. calaba and. The reproductive phenology was studied in one provenance each from Australia and Sri Lanka with relative variation in flowering periods, number of flowers per inflorescence, small or large fruits, mature fruits per cluster and kernel weight per hectare (Hathurusingha et al., 2011). The variation in seed morphometric traits were studied in 12 calophyllum genotypes collected from seven provenances of northern Australia and Sri Lanka (Hathurusingha et al., 2010b). A significant provenance variation in seed length and diameter, kernel weight, exocarp thickness and oil content were reported. A strong correlation was observed between different seed traits; however, seed morphometric traits showed no consistent correlation with the oil content.

The periodic variation in oil content and fatty acid profiles of trees grown in two different fruiting periods in three different provenances of northern Queensland, Australia were reported by Hathurusingha (2011). The low temperature and relatively higher rainfall was found to influence the oil content and fatty acid composition of C. inophyllum oil. The conventional methods such as phenotypic characterization for assessing the diversity are strongly influenced by the environment and hence genetic variation was studied using the molecular markers. The RAPD markers were used to assess the genetic diversity within and inter-population genotypes of C. inophyllum (Nurtjahjaningsih and Widyatmoko, 2011). Five primers were used and 17 polymorphic
loci were identified with 96.95% variation within population. Setsuko et al. (2012) reported the expressed sequence tags based on the simple sequence repeat for assessing the polymorphism in *C. inophyllum* populations in Bonin and Ryukyu Islands, Japan. Forty eight primers were studied with 36 primers showing clear amplification and 23 primers showing polymorphism among the studied populations.

**Pongamia pinnata**

*Pongamia pinnata* L. Pierre (honge) of family Fabaceae is a medium sized glabrous tree, with straight or crooked trunk of 50-80 cm or more in diameter with a spreading crown and drooping branches. Bark is greyish green and turns brown on subsequent growth and attains smooth or faintly vertically fissured. The branching begins to grow above the ground level from 5 to 7ft. The trunk has primary and secondary branches; the primary branches range from two to four. *P. pinnata* is a legume species with tap root system and root nodules resulting from a symbiotic relationship with nitrogen-fixing bacteria. Leaves are imparipinnate with long slender leaf stalk, glabrous, pinkish-red when young, glossy dark green above and dull green with prominent veins when mature. Leaflets are 5-7 paired with a terminal leaflet, ovate or elliptic with short stalk, not toothed at the edges but slightly thickened (Anon., 1969). Inflorescence a raceme, axillary, 5-20 cm long, drooping. Flowers are short stalked; calyx dark purplish-brown: cup-like. The corolla with two white to pink tinged colour wings and two white keel petals and one greenish-white standard petal with strong fragrance. The standard petal is broad with light greenish-yellow nectar at the center and hook like structures at the base (Raju and Rao, 2006). Pods are compressed, woody, indehiscent and yellowish grey when ripe varying in size and shape, elliptic to obliquely oblong with short curved
beak, seeds usually one rarely two, reniform, broad, wrinkled with reddish brown leathery testa. The seed oil (27-40%) contains mainly oleic acid (18:1; 44.5-71.3%) and linoleic acid (18:2; 10.8-18.3%) along with minor quantity of palmitic acid (16:0; 3.7-7.9%), stearic acid (18:0; 2.4-8.9%) and linolenic acid (18:3; 5.5-6.0%) (Bala et al., 2011; Birajdar et al., 2011; Kesari et al., 2010a).

The seed extract inhibit the growth of herpes simplex virus type 1 and type 2 in Vero cells (Elanchezhiyan et al., 1993). Srinivasan et al. (2001) evaluated the anti-inflammatory activity of alcoholic extract of pongamia leaves on model animal rats. The aqueous and alcohol extracts of leaf and fruit possessed antifilarial activity to the cattle parasite (Uddin et al., 2003). Pongamia is being widely used as a medicinal plant, particularly in Ayurveda and Siddha medicine systems of India (Muthu et al., 2006). More recently, it is widely known for its antimicrobial and therapeutic activities. Brijesh et al. (2006) showed that the leaf extract significantly reduced toxin production from *Vibrio cholerae*. The study was undertaken to investigate the anticonvulsant efficacy of pongamia leaf extract in mice (Manigauha et al., 2009). Treatment of rheumatic pain using leaf extract of *P. pinnata*, *Vitex negundo* and *Eucalyptus globulus* has been reported by Shivanna and Rajakumar (2010). Kesari et al. (2010a) reported that pongamia seed oil exhibited maximum antifungal activity to *Aspergillus niger*, *Aspergillus terreus* and *Candida albicans* and maximum antibacterial activity against *Yersinia enterocoli*, *Listeria monocytogens*, *Escherichia coli* and *Salmonella paratyphi*.

The insecticidal and nematicidal activities of extracts from parts of pongamia have numerous applications in agriculture and environmental management. The petroleum ether extract of leaf tissue was reported to have insecticidal activity towards...
American cockroach (Kabir et al., 2001) and other insects (Pavela 2009). George and Vincent (2005) showed that the petroleum ether extract of seed tissue showed anti-mosquito repellent activity. The pongamia oil-cake showed inhibition of reproduction of root-knot nematode Meloidogyne incognita infecting chickpea (Yadav et al., 2005). The effects of pongamia bark, leaf and seed oil on various agricultural pests were studied and among all extracts, methanolic fraction of seed oil exhibited the maximum reduction in growth of Spodoptera litura and leaf extract was least effective (Kumar et al., 2006a).

The secondary metabolites in pongamia have been well documented by previous workers. The presence of bioflavonoids pongaflavanol and funicatachalcone was reported in stem bark (Bandaranayake, 2002; Bimla and Kalidhar, 2004; Yin et al., 2006). Previous investigations on this plant indicated the presence of abundant flavonoids such as furanoflavones, furanoflavonols, chromenoflavones, furanochalcones and pyranochalcones (Simin et al., 1996; Yadav et al., 2004). The use of P. pinnata de-oiled cake as by-products as animal feed supplement have been reported by previous researchers. The detoxification of deoiled cake by various physico-chemical methods has been reported and alkali treatment (1% NaOH) was found to reduce karanjin content to a large extent (Prabhu et al., 2002). This was confirmed by the detoxification of antinutritional components like tannin, karanjin and trypsin inhibitor activity in de-oiled pongamia oil-cake (Panda et al., 2006). The phytic acid content in pongamia seed-cake was reduced by the method of sprouting and oil frying (Vadivel and Biesalski, 2012). The treatment strategies also improved the protein digestibility of oilseed cake and its use in dietary supplement.
Pongamia seed oil has been recognized for its utility as biofuel in the recent times. The physiochemical properties of pongamia oil make it a suitable substitute for fossil fuel. They are partially domesticated plants and cultivation practices are at various levels of development. Among the biofuel plants, cultivation of pongamia on wasteland is advantageous since it is a legume tree and possesses high tolerance to various soil types including alkaline soil. The vegetative propagation through cuttings, and grafting of elite plants could be used to overcome the period of long gestation period in pongamia. Previous investigators have proved the use of biodiesel from pongamia as an efficient substitute for petroleum fuel in diesel engine. Studies were carried out on transesterification of pongamia oil with methanol and sodium hydroxide for the production of biodiesel with fatty acid methyl ester yield of 97 to 98% under standard conditions (Bobade and Khyade, 2012; Naik et al., 2008). The experimental investigations were carried out to examine the performance of CI engines using different blends (B10, B20 and B40) of pongamia biodiesel. It was reported that the pongamia biodiesel performed better to jatropha and neem biodiesel (Rao et al., 2008).

The adaptation of pongamia to a wide range of edaphic and ecological conditions suggested that there might exist a considerable amount of variability that could be exploited for tree improvement and breeding. The tree improvement programs are dependent on the nature and magnitude of variability that existed in the natural population and the level of heritability of desired traits. The extent of pod and seed polymorphism has been found to play an important role in germination, seedling survival and growth (Manonmani et al., 1996). The seed source from different agro-climatic zones of northern Karnataka conditions was evaluated by Patil et al. (2011). They reported
significant differences in seed traits like seed length, width, thickness, seed germination and seedling growth parameters. Kaushik et al. (2007) also showed that variability existed in 40 CPT collected from Haryana state with maximum 100 pod weight of 403.94 g and seed weight of 186.80 g, and maximum oil content of 44.07%.

In a collection of 75 accessions from four districts of Andhra Pradesh, variation in 100 seed weight and seed oil content was also recorded by Mukta et al. (2009a). A total of 123 accessions from different locations of Andhra Pradesh and Orissa were recorded for seed morphometric traits like seed length, seed weight, seed thickness, 100 seed weight and oil content (Sunil et al., 2009a). The variability in fatty acid profiling of 21 selected accessions from Andhra Pradesh with optimum biodiesel traits has been reported by Mukta et al. (2009b). The variation was observed in stearic (1.83 to 11.50%), palmitic (9.25 to 12.87%), oleic (46.66 to 65.35%) and linoleic acids (12.02 to 32.58%). Divakara et al. (2011) also studied variability in 13 pod and seed traits of pongamia CPT collected different regions of Jharkhand, India. Rao et al. (2011) selected 50 CPT from 11 locations of Andhra Pradesh for evaluating the genetic association and variability in seed and growth characteristics. A significant variation in seed morphology and oil content was reported. Sahoo et al. (2011) also reported variation in pod and seed traits of 53 CPT collected from different locations of Orissa. Seeds from Konkan region of Maharashtra were collected and evaluated for seed and pod variability and reported the seed oil content that ranged from 31 to 42% (Raut et al., 2011).

In general, the phenotypic coefficient of variation was higher than the genotypic co-efficient of variation indicating the effect of environment on the selected traits. The 100 pod weight and seed weight showed positive correlation with oil content (Kaushik
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The pod-seed ratio, 100 pod weight and seed weight of CPT from Haryana showed high heritability and genetic gain. The seed and pod weight showed positive correlation with oil content. Sunil et al. (2009a) reported high heritability along with genetic gain for 100 pod weight (97.6%) and oil content (86.7%) for CPT from South East Coastal zone of India covering Andhra Pradesh and Orissa. These reports were further confirmed by results on high heritability exhibited by seed oil content (93%) of CPT from Andhra Pradesh (Rao et al., 2011), while CPT from Orissa showed high heritability and genetic advance for 100 pod weight (96.1%) and seed weight (90.9%) (Sahoo et al., 2011). The CPT from Jharkhand showed high heritability accompanied by high genetic advance for 100 pod weight (98.4%) and seed weight (96.9%) and positive correlation between 100 pod weight and seed weight with plant height, collar diameter and volume index. Therefore, these traits could be used as the criteria for the selection of candidate plus trees.

The genetic markers give an unbiased result as compared to the morphological and protein markers. Several DNA based markers have been widely used to assess the genetic diversity. The DNA markers include RFLP (Lu et al., 1996), RAPD (Williams et al., 1990), AFLP (Vos et al., 1995), ISSR and SSR (Zietkiewicz et al., 1994) with wider application particularly, the genetic diversity. The efficiency, high polymorphic information and reproducibility of AFLP markers have resulted in its application in genetic diversity studies.

The RAPD analysis was performed to study the genetic relationship between nine constituent species belonging to five traditionally recognized genera including genus Pongamia under the tribe Millettieae. The genus Pongamia exhibited close genetic
similarity with *Millettia* with similarity of 24.83% (Acharya et al., 2004). Kesari et al. (2010) studied the marker systems like RAPD, AFLP and ISSR to assess the genetic variability and relatedness among 10 CPT of pongamia and showed high efficiency of AFLP when compared to other marker systems. The polymorphism levels of 10.48, 10.08 and 100% were achieved using 18 RAPD, 12 ISSR and four AFLP primer combinations, respectively. Thudi et al. (2010) reported the genetic diversity in 48 *P. pinnata* accessions collected from six different states of India. The AFLP primer informativeness for five primer combinations was used to assess the marker utility. The E-ACG/M-CTT primer combination showed highest polymorphism of 98.21%. Accessions showed heterogeneous grouping with respect to the region of collection. Sharma et al. (2010) reported the application of three endonucleases to study AFLP (TE-AFLP) for genetic diversity by comparing the conventional method of using two endonucleases. Twenty individual *P. pinnata* accessions from Delhi were assessed using four primer combinations. The marker attributes showed higher values for AFLP than in TE-AFLP, while the band intensities across different lanes were uniform for TE-AFLP. The ISSR markers were used to study the genetic diversity among seven populations of *P. pinnata* obtained from three different agro-ecological zones of Orissa. The modest level of polymorphism of 67.18% was obtained in pongamia germplasm collection showing within-population variation of 32.34% and polymorphism at species level of 94.3% (Sahoo et al., 2010). These diverse accessions identified in different studies across India could be useful in *P. pinnata* improvement for biodiesel production. Kesari and Rangan (2011) reported the changes in seed moisture content, fresh weight, length, breadth and thickness, total seed protein content and ultrastructural changes occurring at seven seed
developmental stages from pongamia tree in Guwahati. However, no reports are available on the histological, histochemical and biochemical changes occurring during the seed development to predict the harvest time in pongamia.

**Influence of seed fatty acid composition on biodiesel quality**

The better understanding of the structure of fatty acid is of great importance when choosing the oil for desired biodiesel quality. The structural properties of fatty acid that influence the biodiesel quality are the chain length, degree of unsaturation and branching in the fatty acid. The physico-chemical properties of biodiesel that are influenced by the fatty acid composition are ignition quality, heat of combustion, cold flow, oxidative stability, exhaust emission, viscosity and lubricity (Muniyappa *et al*., 1996). The oxidation stability of biodiesel is mainly dependent on the double bonds in the fatty acid chains. Generally, cetane number, heat of combustion, melting point and viscosity increase with increase in chain length and decrease with increase in unsaturation. It is therefore, reasonable to enrich certain fatty acids with desirable properties in fuel such as oleic acid that exhibited a combination of these fuel properties.

The unsaturated fatty acid in oil makes it to remain in liquid form; however the use of polyunsaturated fatty acid for biodiesel production results in poor performance of the engine in cold. The cetane number is the measure of interval between the injection of the fuel into the cylinder and the onset of ignition. The monounsaturated fatty acids caused low cetane number, while saturated fatty acids caused high cetane number (Knothe *et al*., 2003). The higher the cetane number, better is the engine performance and this minimizes the smoke formation due to incomplete combustion (Meher *et al*., 2006).
However, saturated fatty acid forms bent triglyceride structures and these have high melting point causing rapid crystallization at low temperature leading to clogging of fuel engines (Knothe et al., 2005). Higher saturated fatty acids in oil increases cloud point and cold filter plugging points have a negative effect on biodiesel (Ramos et al., 2009). Salimon and Salih (2009) reported that short to medium chain saturated fatty acids form crystalline structures by uniform stacking of the bend triglycerides at low temperature. These crystals lack easy flow due to less kinetic energy. The polyunsaturated fatty acids in biodiesel form polymers which blocks the fuel engines of a vehicle. The viscosity and density are important properties of biodiesel. The viscosity of biodiesel increases with the chain length in fatty acid. The density of biodiesel increases with decrease in chain length and increasing number of double bonds (Refaat, 2009). Usually, the unsaturated fatty acids in oil exhibited better lubricity than the saturated fatty acids.

Saponification value is the chain length of fatty acid in oil, while iodine value represented the degree of unsaturation. The autoxidation of unsaturated fatty acids depended upon the number and position of double bonds. The linoleic acid (one bis-allylic position at C-11) and linolenic acids (two bis-allylic positions at C-11 and C-14) are more prone to oxidation. Ramos et al. (2009) studied the effect of fatty acid composition of various vegetable oils on the biodiesel property. The vegetable oils rich in linoleic acid and linolenic acids such as soybean, sunflower and grape seed oil showed poor oxidation stability, while palm, olive and almond oils showed high stability. Therefore, the oil with monounsaturated fatty acid content is considered favorable for biodiesel production than the polyunsaturated oil with linoleic and linolenic acids of triglycerides stored in the form of oilbodies in seeds. The functional genomics of oil
biosynthesis has been studied in both legumes and non-legume crops by Fait et al. (2006). In addition to this, Wang et al. (2001) and Weber et al. (2005) carried out the seed development with respect to oil accumulation and other storage reserves. Haathurusingha et al. (2010a) reported that saponification value of biodiesel produced from *C. inophyllum* oil at different developmental stages decreased, while the iodine number increased.

**Biochemistry of seed development**

Seeds play an important role in the human welfare, with their utilization as food for animals and humans, medicine and other useful products. Consequently, the information regarding their nutritive value and chemical composition during seed development and germination, harvesting period with maximum physiological maturity and storage changes are the major area of research. The success of agriculture system is mainly dependent on the availability of high quality seeds with good genetic and vigour potential. The seed development is a complex phenomenon essential for the survival of angiosperms. Hence, the process of seed development and the related mechanism becomes the subject of research, in particular the seed filling phase. The induction of flowering and differentiation of flowering parts are the initial steps in seed development (Vicente-Carbajosa and Carbonero, 2005). The process of seed development is controlled genetically and involves a sequence of morphological, physical, physiological and biochemical changes starting from the ovule fertilization to seed maturity (Wobus and Weber, 1999).
The angiosperm seed development proceeds through embryo morphogenesis, seed filling and acquisition of tolerance to desiccation, growth arrest and the dormancy period broken upon germination (Bewley and Black, 1994). The success of early germination and seedling survival is largely determined by the physiological and biochemical features of the matured seed. The reserve food compounds include storage proteins, oil (often triacylglycerols), carbohydrates and traces of mineral nutrients which accumulate during the seed filling stage (Gallardo et al., 2008). The storage compounds contribute up to 90% of the seed dry weight. The embryo and endosperm are covered by a protective covering called the seed coat derived from maternal integuments.

Legume seeds are the major source of food in many of the developing countries. The most common legumes used to study the seed reserve accumulation are Glycine max, Pisum sativum, Vicia faba and Medicago truncatula. The tree legumes are the preferred species for biodiesel feedstock which could be grown on marginal lands with low cultivation operations (Biswas et al., 2011). They can also be grown on nitrogen and phosphorus deficient soils without competing with food crops. However, the literature is lacking on the seed development in tree legumes. The principal storage organ of legume seeds is the cotyledons. Because of their nutritional and economic importance, efforts are being made to study the biochemical and molecular processes underlying the seed development. Nutrients from phloem of the vegetative parts are loaded into the seed coat. During the filling stage, a transient accumulation of reserve compounds like starch, sucrose and amino acids occur in the seed coat (Gallardo et al., 2008).
Stages of seed development

The development of seed could be divided into three phases according to the dry matter accumulation. Initially, the seed development is accompanied by slow biomass accumulation during embryogenesis. The maturation phase is accompanied by a fast accumulation of dry matter that coincided with the reduction in seed fresh matter until complete physiological maturity is reached. In this phase, the cell expansion and reserve material accumulation takes place leading to the growth of embryo or cotyledon. The third phase is characterized by seed dehydration together with mechanisms related to embryo desiccation tolerance and germination viability. Sugars and nitrogen are signals regulating the seed development (Wobus and Weber, 1999). The seed coat is involved in the metabolic exchange between the seed and vegetative parts of the plants. Hormones are involved in different phases of seed development from the beginning of embryo development to desiccation tolerance at maturity. The cytokinin which is present during initial cell division process, diminishes as the seed matures. Auxins are present from the beginning of seed development and are responsible for reserve material accumulation. Gibberellins are associated with cell expansion during the second phase of seed development. Abscisic acid is involved in the synthesis of late embryogenesis associated protein during the desiccation phase (Ali-Rachedi et al., 2004; Koornneef et al., 2002).

Stage I: Embryogenesis

The early stages of seed development involve fertilization, zygote formation and intense metabolic activity with high demand for low molecular weight precursors like sucrose, amino acids, fatty acids, nucleosides, organic acids, water and inorganic ions.
This phase is initiated by cell division and differentiation that forms the embryo and endosperm tissue. The embryo develops into different forms during the process of seed development. In most dicots, the developing embryo feeds on reserves in the endosperm and cotyledons develop into large and fleshy first leaf that store reserve food material for seedling growth. The process of embryo development is also accompanied by the modification of integuments leading to the formation of seed coat (Noggle and Fritz, 2002).

In legumes, the growth of embryo confined within the seed coat is related to the crushing of the inner seed coat cell layers (Weber et al., 1995). In general, the outermost layer of seed coat is the epidermal layer, which is a single layer of palisade cells (macrosclereids). The next cell layer is the hourglass cell layer which is composed of thick-walled osteosclereids. The innermost cell layer of seed coat is multi-cellular layer of partially flattened parenchyma. Immediately placed inside the inner parenchyma cell layer is the aleurone layer. Each of these layers is derived from the maternal outer and inner integuments. The next layer is the endosperm layer which is tightly compressed due to the expansion of cotyledon cells (Miller et al., 1999). The seed coat of legumes are relatively large and complex, controlling the aqueous and gaseous environment around the embryo and protection against pests and diseases (Ndakidemi and Dakora, 2003; Souza and Marcos-Filho, 2001). Both the palisade and hourglass cell walls play an important role in the mechanical strength of the seed coat (Van Dongen et al., 2003). The chlorenchyma and parenchyma cells in the seed coat may be involved in the post-phloem transport of nutrients during the seed development. The aleurone cell layer is known for its role in the enzymatic mobilization of seed reserve material during germination (Ma
et al., 2004). Generally, in legumes, the seed development has been reported featuring changes occurring in pod wall, seed coat, endosperm and embryo. During embryogenesis, numerous starch granules in hourglass cells indicate that the seed coat could synthesize nutrients for the developing embryo (Wang and Grusak, 2005). In legume seeds, the developing pod walls and seed coat are transient reserves of assimilates and other nutrients for transportation into the developing cotyledons (Borisjuk et al., 2003; Moise et al., 2005).

During the pre-storage phase in embryo development, the sucrose in the seed coat is transferred unaltered but it is later hydrolyzed by extracellular invertase enzyme. During seed development in faba bean seeds, cell wall bound insoluble invertase in the seed coat promoted the embryo cell division (Weber et al., 1995). The electrical conductivity of the developing seeds is mainly dependent on the lignin content of seed coat (Panobianco and Vieira, 1996). During the seed coat development, the mineral nutrients, especially the potassium ions are involved in cell turgor pressure and involved in sucrose unloading from the seed coat to developing embryo. The high hexose to sucrose ratio is believed to promote embryo growth by enhancing the cell division (Borisjuk et al., 1998). The seed developmental changes in secondary thickenings of palisade cell layer in soybean seed coat are reported by Miller et al. (1999). The amino acids imported through phloem from vegetative parts undergo elaborate metabolic conversion in the seed coat before being released into the apoplast (Van Dongen et al., 2003). Various histochemical reports suggest the presence of lignin, tannin and other phenolics in the seed coat layer during seed development. Taiz and Zeiger (2006) reported that lignin is associated mainly with the hemicellulose in seed coat layer.
ensuring the protective survival of the offspring by maintaining an environment around
the embryo during extreme conditions. Villavicensio et al. (2007) reported that at
different seed moisture content levels, the hydrophobic lignin content in palisade layer of
seed coat makes it impermeable and hard-seeded, and with decrease in moisture content
the seed becomes harder. The browning of the seed coat at the later stage of seed
development could be attributed to condensation of tannins as reported in other legume
seeds by Pinzon-Torres et al. (2009).

Stage II: Storage compound accumulation

Phase II is the period when the embryo stops growing, low cell division and
maximum seed dry weight increase is observed (Gutierrez et al., 2007). The low
molecular weight precursors are used up by the developing seed for the synthesis of
storage reserve materials. The leaf sucrose is the major carbon skeleton for most of the
storage compounds including nitrogenous constituents like amino acids, amides and
nucleotides. In legume seeds, the capacity of cotyledons to accumulate dry matter content
could be dependent on the final cell number (Munier-Jolain et al., 1998).

Generally, monosaccharides are high during the initial stages of development and
oligosaccharides are high at the later stages of development (Blackman et al., 1992).
During the initial phase of seed filling, a high rate of sucrose transport into the embryo
takes place which promotes storage activities (Panitz et al., 1995). Oligosaccharides play
an important role as reserve polysaccharides in plants and are typical of the endosperm of
a wide range of legume seeds (Buckeridge et al., 2000). Golombek et al. (2001) reported
that in legume seeds simple molecules are transported to the developing seeds such as
sucrose, amino acids and mineral ions. Sucrose synthase in the seed coat is associated with the starch synthesis in the cotyledon (Rosche et al., 2002). Carbohydrates are discharged from seed coat vascular system to an apoplastic region between the seed coat and the embryo (Borisjuk et al., 2003). Djemel et al. (2005) reported that Medicago truncatula synthesizes oligosaccharides like raffinose and stachyose at mid-maturation, while sucrose content decreased sharply. The sucrose induces the transcriptional regulation of several enzymes like phosphoenolpyruvate carboxylase which correlated with seed protein content (Weber et al., 2005). The sucrose reaches seeds through the ventral vascular system located in the pod in the hilum region in the seed coat. Seed coat sucrose and amino acid transporter have been reported in legume, which controls the flux of reserve accumulation in the developing seed (Tegeder et al., 2007, Zhou et al., 2007). Sharma et al. (2011) reported a gradual decrease in soluble sugars during seed development in soybean.

Kang and Rawsthorne (1994) reported that during both fatty acid and starch synthesis, glucose-6-phosphate is one of the precursors in the initial step of seed biosynthetic pathway in plastids. King et al. (1997) reported that during early stages of seed development, starch synthesis is accompanied by the accumulation of photosynthetic proteins like chlorophyll protein, oxygen evolving enhancer protein and ribulose-1, 5-bisphosphate carboxylase/oxygenase (RiBISCO) small subunits. These help in recycling the CO₂ released during fatty acid synthesis as a source of carbon which further furnish the oil biosynthesis during later stages of oilseed development. The starch granules within plastids of developing peanut have also been reported by Young et al. (2006). In these plastids, the association of starch grains with thylakoids might indicate
that this structure would result in chloroplast formation during germination. The electron-dense region within the developing starch grains of plastids might provide evidence of the location for enzymatic activity during the biosynthesis of starch. These starch reserves provide a sugar source during seed germination.

The protein content of legume seeds varies from 20 to 40%. The major storage protein in legumes are 7S (vicilin) and 11S (legumin) globulins. In general, the total protein content of legume seed includes storage proteins, house-keeping proteins, biologically active enzymes, protease inhibitors, lectins and allergens. In legumes, the storage protein is sequentially controlled during seed development. It has been reported earlier that the oleosin corresponds to protein subunit band between 20 and 19 kDa (Frandsen et al., 2001; Katavic et al., 2006). In M. truncatula, the vicilin is produced first followed by legumin. Globulin contributes to 80% of the total seed protein content. The accumulation of seed storage protein is independent of nutrient availability and metabolism during seed filling, although it is genetically controlled (Gallardo et al., 2008). The low molecular weight protein bands (6 to 30 kDa) at later stage of seed development were reported in Olea europaea (Wang et al., 2001) and Lesquerella fendleri (Chen et al., 2009).

The crystalloid structures are present in the protein bodies at the later stages of seed development. Globoids are electron-dense inclusions that mainly constitute mineral reserves such as iron, manganese, magnesium, potassium and calcium (Lott, 1981). Phytoferritins are the protein storage units in the cell which are present in the early stage and absent in subsequent stages of development in Vicia faba (Johansson and Walles, 1994). The ultrastructural studies of developing seeds have shown that protein bodies
arise from the endoplasmic reticulum or golgi vesicles and are sequestered into vacuoles to form PSV (Herman and Larkins, 1999).

Glutamine, asparagine, arginine and glutamic acid are abundantly found in the young developing seeds. S-adenosylmethionine synthesized from amino acid metabolism in seeds is the methyl group donor for methylation of amino acids, lipids, DNA and RNA and regulates the synthesis of threonine, lysine and methionine (Ravanel et al., 1998). In developing soybean seeds, the level of asparagine plays a rate limiting role in storage protein accumulation (Hernandez-Sebastia et al., 2005). Glutamine acts as a precursor for the synthesis of Proline which accumulates during drought and salinity, low and high temperature and in presence of heavy metals (Forde and Lea, 2007). The amino acid composition of developing seed depends on the temperature, nitrogen availability in soil and remobilization of nitrogen from vegetative parts (Carrera et al., 2011).

The storage lipids in many oilseeds are usually in the form of triacylglycerols (TAG) which are esters of glycerol with fatty acids. The TAGs are embedded in subcellular structures called oilbodies. The size of oilbodies is regulated by the ratio of oil to oleosin (Tzen and Huang, 1992). The oilbodies are surrounded by an electron-dense layer made up of oilbody associated proteins called oleosin along with caleosin and single layer of phospholipids (Frandsen et al., 2001). Borisjuk (2005) and Hsieh and Huang (2004) reported the ultrastructural evidence for the presence of endoplasmic reticulum and plastids during the genesis of oil bodies. The carbon in fatty acids is in the reduced form resulting in the release of high energy during germination than the other storage compounds (Graham, 2008). In oilseeds, the TAG is synthesized by multiple interconnected pathways at cellular organelles. The rate limiting reaction of TAG
biosynthesis is catalysed by diacylglycerol acyltransferases (DGAT) leading to the increased oil content (Bates et al., 2009; Lung and Weselake, 2006). The ultrastructural studies have shown unusually large oil bodies in low oil content seeds and small oil bodies in high oil content seeds of brassica cultivars (Hu et al., 2009).

Rahamatalla et al. (2001) reported that during early stages of seed development in safflower, the triglyceride accumulation was very slow. Sorensen et al. (2005) reported that in flax seed, the oil content increased on dry weight basis with simultaneous decrease in linoleic acid until 20 days after flowering and then remained constant. The palmitic acid content decreased in safflower 15 days after flowering, whereas oleic and linoleic acids increased until maturity (Gecgel et al., 2007). Oleic, palmitic and stearic acids are the major components of cell membrane and their presence was important during the early development stage of cotyledon (Voelker and Kinney, 2001). The de novo synthesis of palmitoyl-ACP occurs initially, followed by its elongation to stearoyl-ACP, at this point of time when the steady decrease in palmitic acid content is observed during seed development. Stearoyl-ACP is then desaturated to oleoyl-ACP which is later in turn desaturated to linoleoyl phosphatidylcholine. The elongation and oleate desaturation activity in desaturation step plays an important role in regulating the relative amounts of 16-carbon and 18-carbon fatty acids in oilseeds (Wu et al., 2009). Many physical and physiological factors like energy, oxygen, embryo photosynthesis and temperature contributed to the seed oil production (Baud and Lepiniec, 2010).

Minerals are important part of seed development since they are required during several cellular processes and support the growth and early development of seedling. Grusak and DellePenna (1999) reported that several mineral nutrients are stored in
Medicago truncatula seeds and most of them are delivered to the seed coat by phloem. These minerals are subsequently distributed to the embryo by apoplastic method (Lalonde et al., 2003). Generally in oilseeds, one third of the carbon in the sucrose is lost by decarboxylation in fatty acid synthesis (Alonso et al., 2001; Schwender and Ohlrogge, 2002). This carbon is refixed for lipid biosynthesis during the early phase of seed development (Ruuska et al., 2004). Carbon and nitrogen are imported in the form of organic compounds from roots to vegetative parts and its remobilization during seed development. Carbon from sucrose enters the storage metabolic pathway by the activity of invertase and hexokinase during the seed development (Tomlinson et al., 2004).

During legume seed development there occurs a simultaneous increase in the dry matter, total protein content and nitrogen content (Golombek et al., 2001). Asparagine and glutamine are the two forms of nitrogen supplied to the developing seeds. Miranda et al. (2001) reported that in faba bean cotyledons, a co-transport of aminoacids with H⁺ are regulatory points during protein storage phase of seed development. The storage reserve accumulation is also controlled by the genotype and environment in which the plant species is growing. Sulfur compounds like sulfate, S-methyl methionine and glutathione influences the seed reserve accumulation by modifying the legumin to vicilin ratio (Tabe and Droux, 2001). In legumes, nitrogen is supplied to the developing seed through the phloem in the form of amino acids and amides for the synthesis of storage proteins (Weber et al., 2005). The nitrogen accumulation by seeds during filling depends upon the soil mineral nitrogen supply, symbiotic fixation of atmospheric nitrogen and endogenous nitrogen accumulated in the vegetative parts (Schiltz et al., 2005). The nitrogen content of seed at maturity is helpful in seedling growth and development at low
soil fertility habitats (Naegle et al., 2005). Delgado-Alvarado et al. (2007) reported that phosphoenolpyruvate carboxykinase in developing legume seed is strongly influenced by the nitrogen supply both in organic and inorganic forms thereby indicating its involvement in nitrogen metabolism. Gotz et al. (2007) reported that in transgenic *Vicia narbonensis* accumulation of nitrogen occurred only at later stages of seed development with simultaneous increase in seed dry weight due to over-expression of enzyme amino acid permeases.

Eighty per cent of total seed phosphorus is stored in the form of phytate. The phytate crystals of manganese, zinc and iron crystal complexes are stored in the protein bodies (Otegui et al., 2002; Welch, 1997). The phytate complex is a stable compound stored in seeds resulting in decreased bioavailability of essential minerals to humans and animals which lack the hydrolytic enzyme phytase in their digestive tract. It has been reported previously that during phytic acid biosynthesis, variation in the availability of inorganic phosphorus, myo-inositol and mineral ions might result in variation in the accumulation of phytate at maturity (Raboy, 2003). In recent years, the phytic acid is considered beneficial medicinally to prevent kidney stone formation, diabetes mellitus, atherosclerosis, coronary heart disease and a variety of cancers (Greiner et al., 2006). Therefore, it becomes important to study the accumulation of phytic acid and phosphorus during seed development. The phytate is an important source of inorganic phosphorus, trace elements and myo-inositol which are required during early seedling development stages and during germination. Vadivel et al. (2012) reported 1.8% phytic acid in pongamia seeds collected from Tamil Nadu.
Zinc, manganese, copper, iron and potassium are the other mineral nutrients constituting less than three per cent of the seed dry biomass and form important essential nutrients. Zinc is used by plants in the stabilization of many structural motifs found in transcriptional regulatory proteins (Fox and Guerinot, 1998) and it protects the germinating seeds from soilborne pathogens. During the seed development, minerals are necessary for many cellular processes, growth and early development of seedling. Mineral ions are delivered to the developing seeds by the non-protein amino acid chelating agent called nicotianamine (von Wiren, 1999). Many enzymes require zinc for their integrity and function. Manganese is a major component of enzyme Mn-superoxide dismutase in seeds. The manganese in PS II complex of photosynthetic electron transport chain coincides with high chlorophyll content of developing seeds (Otegui et al., 2002). Ozturk et al. (2006) reported that zinc concentration decreases as the seed attains maturity in Triticum aestivum.

Laszlo (1991) showed that the iron and chlorophyll content occur parallely and decrease during later stages of soybean seed development. In developing seeds of Glycine max, the potassium content remained constant throughout the seed development (Laszlo, 1994). The chlorophyll content increased during active metabolism and declined during maturation. The potassium ions are involved in cell turgor pressure and in sucrose unloading from seed coat to the developing embryo. Copper is mainly stored in plastids and involved as a component of plastocyanin in photosynthetic electron transport chain (Maksymiec, 1997). Iron is an important co-factor for many of the redox reactions involved in photosynthesis, respiration and formation of chlorophyll. Samarah and Ereifej (2009) reported that in developing seed of Vicia sativa manganese and zinc decreased as
seeds matured, while phosphorus, iron, potassium and copper remained constant throughout. In mitochondria, iron is required for Fe-S cluster for electron transport chain. Iron must be bound to storage protein embedded in vacuoles to prevent cellular damage (Conte and Walker, 2011).

**Stage III: Desiccation**

The desiccation phase begins with the arrest of seed growth and seed enters into dormancy with complete seed vigour (Harada, 1999). The sub-cellular organelles such as mitochondria and chloroplasts were found to disintegrate during the later stages of seed development. This reduction in sub-cellular membrane surface appears to be the mechanism to slow down the cellular metabolism and prevent the physical damage to the internal membranous system of cell (Vertucci and Farrant, 1995). The ultrastructure studies have shown that the oil bodies at the later stage of seed development tend to experience cytoplasmic compression but following resistance to aggregation, they remain as individual entities (Frandsen et al., 2001). Cherif et al. (2004) showed decrease in moisture content of almond cultivars during the later stages of seed development. The desiccation tolerance in developing seeds is associated with the increase in distinct sugars, organic acids and certain metabolites (Fait et al., 2006). Borek et al. (2009) also reported the decrease in moisture content with simultaneous increase in dry matter and decrease in fresh matter in developing seeds of lupin species.

High ratio of phosphatidylcholine to phosphatidylethanolamines accumulates during the desiccation phase to prevent damage to the membrane bilayer structure (Vertucci and Farrant, 1995). Stress tolerance sugars are synthesized at the late stage of
seed development impairing membrane fusion and increase seed longevity (Gurusinghe and Bradford, 2001). During the late embryogenesis, abundant proteins characterized by hydrophilic amino acid composition are highly soluble in water and protect seeds to heat and other environmental stress during the desiccation phase (Wang et al., 2003). The increase in non-reducing sugars at the later stages of seed development has been reported in legumes (Djemel et al., 2005). The transcriptome analysis of the developing seeds of *Medicago truncatula* showed up-regulated activity of genes for late embryogenesis abundant proteins, heat shock proteins, antioxidants and down-regulation of many genes involved in primary metabolism and cell cycle (Buitink et al., 2006). During desiccation, abscisic acid caused degradation of chlorophyll in the developing seed (Nakajima et al., 2012).