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In Vitro Propagation of Potential Biodiesel Plant, *Pongamia Pinnata (L.)* Pierre

Abstract

Pongamiapinnata has recently been recognized as a viable source of seed-oil for production of biofuel to meet the demand of the burgeoning biofuel industry. Although this tree is propagated by seeds, germination and plant vigour decreases following storage of seeds for 3 months or more. Plantspropagated through stem cuttings are not deep rooted, hence not a preferred method of cloning thisvaluable species. Hence, the aim of the present study was to develop an efficient and reproducible masspropagation protocol for raising clonal garden of P. pinnata through in vitro cotyledonary node cultureand assessing its trueness to parent plant.

Keywords: Bioenergy, Biofuel, Biodiesel, Renewable, Feedstock, Yield, Biomass, Maize, Oilseed Rape, Extraction, Phase Separation.

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Introduction

Biodiesel is an alternative fuel for diesel engines produced by Tran's esterification reaction of vegetable oil or animal fats with an alcohol such as methanol, using a base or acid catalyst. However, the production cost of biodiesel is not economically competitive with petroleum based fuel, as the biodiesel feed stocks are costly. Attempts on increasing the rate of biodiesel production have been made. There are many advantages with blending of biodiesel with fossil fuel namely

1. Reduction in emission.
2. Increased efficiency of engine.
3. Low consumption of fuel.
4. Low engine wear, etc.

Tests on combustion engine show the use of biodiesel in diesel engines results in substantial reductions of unburned hydrocarbons (67 %), carbon monoxide (48 %), and particulate matter (47%).

Biodiesel is the first and the only alternative fuel to succeed the evaluation of emission results and potential health effects submitted to the U.S. Environmental Protection Agency (EPA) under Clean Air Act Section 211 (b), this is also a major reason to promote the use of biodiesel in many countries (Halek et al., 2009). In India, 51 % of the fuel is utilized only for transportation purposes and biodiesel can be used as an alternative fuel in heavy trucks, tractors, city transportation buses, farm equipment's can be used with increased blending. India being an agricultural country with vast vegetation, land availability and suitable climatic conditions amicable to grow many plant varieties in waste / degraded lands in arid regions.

The status of India is to approach for biofuel production particularly biodiesel should be without competing with land and water resources which are much needed for food production, while developing wastelands which is unsuitable to grow agricultural crops. Based on extensive research, over 300 diverse species of trees are identified which yield oil bearing seeds; out of these, 37 species were found to be appropriate for conventional biodiesel production throughout the world (Azam et al., 2005 and Subramanian et al., 2005). *Pongamiapinnata*is considered as one of the prominent species which has good yield and high oil content and well adapted to arid and semi-arid conditions (Wani et al., 2009).

Pongamiapinnata (L.) Pierre is a fast growing, glabrous, deciduous tree commonly known as Indian beech, pongam tree and karanja tree, which is a smember of the subfamily Papilionideae more specifically the tribe Millettieae. It is indigenous to the Indian subcontinent and South East Asia, it has been successfully introduced to humid and tropical regions of the world (Scott et al., 2008). It is tolerant to a wide range of abiotic stress like drought, frost, heat, salinity, etc. It grows fast and mature after 4-7 years, yielding fruits which are flat, elliptical, long and each fruit contains 1-2 kidney shaped brownish red kernels. The Pongamia seed contains about 30-40 % oil and have similar properties to that of petro-diesel have gained the importance as biodiesel which is a fast emerging, viable alternative to fossilfuel.

Pongamia can grow on most soil types ranging from stony to sandy to clayey and grows to an elevation of 1,200 m (Meher et al., 2004). The plant thrives well in areas having an annual rainfall from 500-2,500 mm, mature trees can withstand water logging, resistant to high winds, drought, and salinity but are susceptible to freezing temperature (Gerpen, 2005; Karmee and Chandha, 2005 and Walker, 2009) and does not grow well on dry sands. Pongamia is commonly found along waterways or seashores, with its roots in fresh or salt water and grows well in full sun or partial shade. The seedcake is non- edible and it can be used as an organic fertilizer which is rich in nitrogen and micronutrients (NOVOD, 2009). Pongamia oil has high amount of monounsaturated fatty acids which can be highly converted to biodiesel.

Neem grows almost on all types of soils including clayey, saline and alkaline conditions, it is a fast growing plant with long productive life span of 150-200 years, it has ability to grow and survive in drought and poor soil and also at a temperature of 4-40 ^{0}C. Neem oil contains steroids and triterpnoids of which Azadirachtin in commonly known. Neem oil has high molecular weight, viscosity, density, free fatty acid value and flash point which is accompanied with moisture content and some impuritites (Bhandare and Naik, 2015).

Simarouba is a medium sized tree attains a height of 20 m and has a life span of 70 years. It grows in a wide range of agro-climatic conditions like warm, humid and tropical regions. The condition depends upon rainfall distribution and water holding capacity of the soil. The seeds contain about 40-50 % oil and yields about 3-4 tonnes of seeds per hectare every year. Simarouba is rich source of fat having melting point of about 29 ^{0}C (Mishra et al., 2012)

Aim of the Study

Status of the vehicular pollution Control program in India.

Materials and Methods

Sample Collection and Extraction of Oil

Pongamia, Simarouba and Neem seeds were procured from Biodiesel Technology Park, University campus and were shade dried at ambient temperature for two to three days to attain equal weight and moisture content. The seed oil was extracted by a mechanical expeller and the filtered oil was used for testing the biochemical properties of oil and for production of biodiesel by Tran's esterification process.

Free Fatty Acid (FFA) = MBR X 28.2X 0.1/ weight or volume of sample

Where MBR is mean burette reading; 0.1 is Normality of NaOH; 28.2 is mol. wt. of standard oleic acid divided by ten. For 1 FFA value, 4.5 gm of NaOH in 30 % of methanol (sodium methoxide) is used for the reaction.

Production of Biodiesel by Transesterification Reaction

The transesterification reaction was performed in a three necked flask, the reaction was performed using 1:3 ratio of lower alcohol (methanol) and oil in presence of acidic or a basic catalyst based on the value of free fatty acids in oil i.e. <0.1 % (w/v), the reaction is base catalyzed transesterification process and value of free fatty acids in oil>0.1 % (w/v) by acid transesterification method (Vivek, 2004; Karmee and Chandha, 2005 and Onkar et al., 2010).

Acid Catalysis

Transesterification reaction by acid catalysis for production of biodiesel is made by calculating the required amount of concentrated sulfuric acid and methanol to neutralize the free fatty acid followed by base catalysis reaction.

Calculation

Quantity of Methanol

2.25 X FFA = x/0.7914 = y ml/liter of oil

y ml X density of oil = z ml of methanol/liter of oil

Quantity of Conc. Sulfuric Acid

0.05 X FFA = x/1.84 = y ml /100 gm of oil

y ml X density of oil = z ml H_2SO_4 of /liter of oil

Where 2.25 is factor for methanol calculation; 0.7914 is the density of methanol; 0.05 is factor for conc. sulfuric acid; 1.84 is density of conc. sulfuric acid and FFA is free fatty acid.The oil is heated at 65 ^{0}C with constant stirring; the calculated mixture of methanol and conc. H_2SO_4 (sodium methoxide) is carefully mixed and allowed to react for about 90 min. till a brown colored layer is formed as an upper layer. The mixture is cooled and if the FFA of the oil is noted below 0.1 % (w/v), the reaction will be followed with base catalyzed steps for biodiesel production.

Base Catalysis

The reaction was performed in three necked flask, the oil is heated at 65 ^{0}C with constant stirring, catalyst reaction mixture (sodium methoxide) was added slowly and allowed to react till the reaction turns brick red color (approx. 2 hr). The mixture was separated in a separating funnel [kept overnight (atleast for 6 hrs) undisturbed], two layers separate as lower layer contains glycerol, un-reacted sodium methoxide, methanol, etc and the upper biodiesel or methyl ester layer is further processed for washing with warm water to separate the unreacted water soluble reagents. After washing, the biodiesel is heated at 110 ^{0}C to remove the moisture content. The quality of biodiesel is analyzed after drying and the yield of methyl ester is calculated using theformula.

$$\text{Yield of methyl ester} = \frac{\text{grams of methyl ester produced}}{\text{grams of oil used in the reaction}} \times 100$$

Quality Testing of Biodiesel

The quality of biodiesel is measured to match the standard specification of the American Society for Testing and Materials (ASTM). The ASTM test methods provide information about the product and ascertain the quality by a standard test results to the users. The test methods are frequently intended for use after satisfactory judging the materials to specification with fundamental properties under laboratory test procedures. It is the most reliable way to ensure that fuel consumers has an access to high-quality fuel. The quality of biodiesel is assessed by the standard laboratory instruments i.e.viscosity, copper strip corrosion and flash point test performed as per standard methods ASTM D445, ASTM D93 and ASTM D130 respectively.

Copper Strip Corrosion Test (ASTMD130)

A known quantity of biodiesel samples were taken in copper strip test bomb and immerse a polished copper strip in the test bomb. The copper strip corrosion test bomb apparatus was kept in a vertical water bath and the temperature is set at 50±1 ^{0}C for 3 hrs. The copper corrosion strip is compared with standard copper strip for any color change as per the standard ASTM D130 methodology.

Viscosity Test (ASTMD445)

Viscosity is a measure of resistance to the rate of flow of liquids/fluids. The viscosity of biodiesel is determined using Tube No. 5 Canon-fenske viscometer, the oil samples were taken in Canon-fenske Tube No. 5 up to the mark on the bulb and kept inside a water bath at 40ºC for a period of 30 min. After 30 min, the biodiesel was allowed to flow through the upper meniscus to reach the lower meniscus on the tube, a stop watch was used to maintain the time required for biodiesel to flow down the mark. The time was noted and the viscosity was calculated according to the formula

Kinetics viscosity (cst) = (time in seconds) X (standard factor of tube 5 bulb in viscometer tube)

Flash Point Test (ASTMD93)

Flash point temperature is a measure of tendency of the test specimen to form a flammable mixture with air under controlled laboratory conditions. Flash point is the measure, which describes a fire hazard or fire risk and response to heat of materials, products or assemblies. The flash point of biodiesel was determined by Pensky Martens open or closed cup apparatus. The cup was filled with biodiesel till the mark (about 75 ml) and the cup was heated at a rate of 5.6 ºC per minute with continuous stirring. The fires sparks were checked using an external flame was passed over the surface of biodiesel. The temperature for flash point is always within the 10 ºC of the probable temperature. When the flash temperature was reached the surface of biodiesel catches sparks, the temperature at the moment was noted and reported as the flash point temperature.

Results

Extraction of Pongamia Seed Oil

Pongamia, Simarouba and Neem seeds were procured from Biodiesel Technology Park, Gulbarga University campus and processed for extraction of oil. Estimation of oil content by soxhlet method is much higher than the oil expeller. The yield of seeds per hectare is noted highest in Pongamia (5-6 tonnes) followed by Simarouba (3-4 tonnes) and in Neem (2-2.5 tonnes). The highest quantity of seeds (kg) for extraction of one liter of oil was observed in Neem (5.7 kg), followed by Simarouba (5.3 kg) and the least was noted in Pongamia (3.9 kg). The estimate of oil content by soxhlet method is about 43 %, but the oil extracted by new oil expellers is about 25-30 %. Thus, one liter of Pongamia oil would need about 3.9 kg of Pongamia seeds and the amount of seedcake produced is 653 gm as presented in Table. The oil extracted from Simarouba seeds is about 40% which also produces 528 gm of seed cake. However,the oil extracted from Neem seeds is about 20 % and seedcake produced is about 438 gm/kg of Neem seeds.

The specific gravity of Pongamia, Simarouba and Neem oil was observed to be 0.91, 0.94 and 0.96 respectively. The free fatty acids (FFA) are the derivatives of oil when they are not attached to other molecules, they are either saturated or unsaturated fatty acids, the estimation of FFA by simple titration method is about 2.24, 3.63 and 4.71 mg/g in Pongamia, Simarouba and Neem oil.

Transesterification Reaction

The amount of biodiesel produced by transesterification of Pongamia oil is 895.96 ml/l and the amount of byproducts glycerin is 126.93 ml and seedcake is 653 gm. The quantity of biodiesel produced from Simarouba oil has a value of 860 ml/l of oil and as of Neem is 873 ml/l of oil. The amount of glycerin/l of biodiesel is studied to be highest in Simarouba (195 ml), followed by Neem (135 ml) and least value was studied in Pongamia (126 ml) as depicted in Table.

Quality Testing of Biodiesel

All the quality tests for biodiesel viz. copper strip corrosion (ASTM D130), viscosity (ASTM D445) and flash point (ASTM D93) were performed by ASTM standard methods and proved to be of good quality according to BIS and ASTM D6751 standards for biodiesel (Table). The copper strip corrosion test of biodiesel was measured as specified by ASTM D130 methodology and no corrosion was observed on the copper strip tested for all the biodiesel samples as depicted in

Viscosity of biodiesel was measured as per the methodology of ASTM D445 and was calculated to be about 4.86 cst for Pongamia biodiesel and 5.68 cst in Simarouba biodiesel and 5.96 cst in Neem biodiesel. The flash point of biodiesel was measured as per the methodology by ASTM D93 and flash point is the measure of a sample to form flammable mixture at a particular temperature under controlled conditions. The flash point of Pongamia biodiesel is observed to be 152 ^{0}C which is stated least as stated in the highest value for flash pointwas observed to be of 165 ^{0}C in Simarouba followed by 158 ^{0}C in Neem biodiesel.

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Traits/Characters	Pongamia	Simarouba	Neem
Density of oil	0.91	0.94	0.97
Free fatty acid (mg/g)	2.24	3.63	4.71
Yield per hectare (tonnes)	5-6	3-4	2-2.5
Seeds/liter of oil (kg)	3.97±0.15	5.23±2.54	5.74±3.73
Oil extraction by expeller (%)	28.53±1.3	40.3±2.3	20±1.76
Amount of biodiesel/liter of oil (ml)	895.96±13.94	860±.10.32	873±15.56
Amount of glycerin/liter of biodiesel (ml)	126.93±16.65	195±2.6	135±4.64
Amount of seedcake/kg of seeds (gm)	653±5.47	528±8.45	438±10.6
Copper strip corrosion	Negative	Negative	Negative
Viscosity of biodiesel (cst)	4.86±0.15	5.68±0.23	5.96±.19
Flash point of biodiesel (^{o}C)	152.4±2.06	165.3±3.7	158. 2±2.23

Table : Comparison Statements of Oil and Biodiesel Quality Parameters of Pongamia, Simarouba and Neem Growing in North Karnataka Region, India

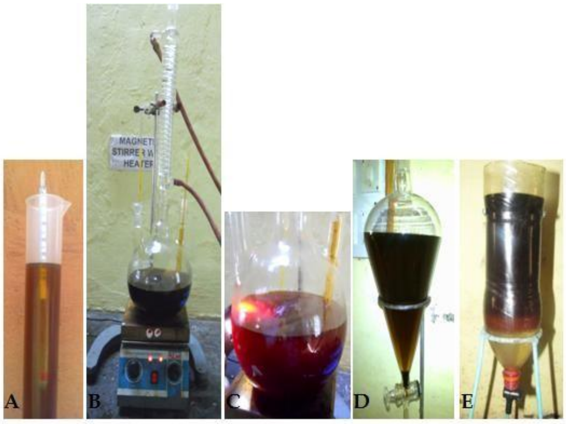

Fig. Properties of Pongamia Oil and Lab Scale Production of Biodiesel from Pongamia oil,

A. Specific gravity test by hygrometer;
B. Transesterification reaction in a three necked flask;
C. Formation of methyl esters as cherry red color;
D. Separation of biodiesel from glycerin after transesterification process;
E. Washing of biodiesel to wash unwanted or unreacted reagents.

Large Scale Production of Biodiesel and Quality Testing of Biodiesel

A. Transesterification unit;
B. Viscosity testing unit;
C. Copper corrosion testing unit;
D. Flash point testingunit

Discussion

Use of biodiesel reduces environmental pollution and the fact that it is made from renewable resources make it more attractive. Biodiesel is prepared by 4 ways 1) Direct use and blending, 2) Micro emulsions, 3) Thermal cracking (pyrolysis) and 4) Transesterificationreaction.

Biodiesel derived from vegetable oil or animal fats is used as a substitute for petroleum diesel mainly because biodiesel is a renewable, domestic resource with an environmentally emission profile and is readily biodegradable. The reaction can be of high quality with the use of enzymes namely lipases. Usage of biodiesel is a replacement to petroleum diesel, which reduces the dependence of many countries on foreign petroleum, it also creates jobs, and it is an eco-friendly fuel. Within a decade of commercial scale production, the industry is proud of its careful approach to growth and a strong focus on sustainability. According to the Environment Protection Agency (EPA), biodiesel reduces greenhouse gases by at least 57-80 % when compared to petroleum diesel making it one of the most practical and cost effective ways to immediately address climate change. Domestic production of biodiesel with natural resources, its use decreases our dependence on imported fuel and contributes to economy of the country.

Biodiesel from *Jatrophacurcas*, *Pongamiapinnata*, *Madhucaindica*, *Simaroubagluaca,* and *Azadirachtaindica*seeds are known to provide a useful substitute for diesel by the transesterification process (Fangrui and Milford, 1999; Fukuda et al., 2001; Suresh et al., 2003; Mishra et al., 2012 and Bhandare and Naik, 2015). The product of incomplete transesterification and incomplete separation may produce biodiesel of low quality. Thus, the reaction should be completed and the glycerol and methyl ester layer should be separated completely and also the flavonoids are to be removed from the product by washing. The amount of biodiesel production depends on the seed weight as it directly depends on oil content in seeds (Shrivastava, 2008). The quality of biodiesel with prime parameters is the viscosity, which controls the characteristics of the injection from the diesel injector. The viscosity of vegetable oil is little higher and it is important to control it within an acceptable level to avoid a negative impact on fuel injector system performance. The reaction of oil and methanol without a catalyst will reduce the water wash step, but the ratio of methanol to oil should be 42:1 and reaction temperature of 300-350 ^{0}C (Saka and Kusiana, 2001). The reaction was also measured without catalyst at 120-180 ^{0}C; this reduced the reaction kinetics (Dasari et al., 2003). The surface reaction will decrease with decrease in the ratio of reactor surface area to volume. The hard formation of glycerin and soap layer contributes to the most appropriate aspect of biodiesel production (Ali et al., 2013). The separated byproduct can give economical support which is one of the advantageous aspects of biodiesel production. Biodiesel is an increasingly attractive, non-toxic, biodegradable fossil fuel alterantive that can be produced from a variety of renewable sources. A pure biodiesel was obtained by in-situ alcoholysis of soybean oil, increased reaction temperature and time and decreasing the particle size of soybeans, the water content of ethanol was also studied by Kildiran et al., 1996. Direct methanolysis of triglycerides using immobilized lipase by flowing supercritical CO_2 (Jackson and King, 1996). To make biodiesel from neem oil, the base catalysed transesterification was selected as the process and methyl esters were obtained in the range of 60-85 % (Bhandare and Naik,2015).

Flash point of a fuel is the temperature at which it ignites when exposed to a flame or spark. The higher the value of flash point, the safer is the biodiesel for transportation. The seedcake obtained is rich in nutritive value and can be used in organic farming, biogas production, etc; glycerin can be converted to pure glycerol and used in food, pharmaceutical or cosmetics industries and the byproduct obtained depends on unsaturated fatty acids in oil. Thermo-chemical conversion of glycerol to propylene glycol by fermentation of *Anaerobiospirillum, Succiniciproducens*for the production of succinic acid is also reported by Dasari et al., 2005 and Alhanash et al., 2008.

In the present study, the comparison on yield, biodiesel production and quality of biodiesel was made, which shows variations in all the studied traits. The highest yield was observed in Pongamia (5-6 tonnes of seeds per hectare), low density of oil, viscosity, free fatty acid content and other traits makes Pongamia as the best plant species for production of biodiesel. Thus, mass propagation of Pongamia can be done for production of biodiesel in semiarid conditions of North Karnataka, India.

Summary and Conclusion

The *Pongamiapinnata*is a promising plant species for production of biodiesel because of it multiple benefits and wide adaptability. Pongamia seed contains 30-40 % oil, grows in arid and semiarid climatic conditions, resistant to abiotic and biotic stress, ability to fix nitrogen and also has capacity to sequester carbon, etc. But, the challenging task as of today is to screen the naturally available wild Pongamia plant resource for the elite plant material with higher productivity via plant improvment program. Pongamia is highly an out-breeding species leading to high variations in plant characters which needs a comprehensive study on variations among inter and intra population levels to exploit Pongamia as a versatile biodiesel plant. In our present thesis work, a detailed study on Pongamia was conducted to study the variations among the Pongamia ecotypes growing in semiarid climatic conditions of North Karnataka region. There is availability of about 2 lakh hectares of wasteland which can be used for plantation of Pongamia, which can produce 400-500 million liters of biodiesel every year and help in bringing economic development with an ecofriendlyapproach.

The emphasize on the general introduction and review of literature about the past and present work on Pongamia, variability studies, plant tissue culture, production of biodiesel and genetic engineering studies in biofuel plant species. The chapter depicts mainly about the concern of using fossil fuels; the importance of using biofuels mainly biodiesel produced by plant species. The necesity for identification of elite trees by tree breeding program using various marker system, plant tissue culture and genetic engineering in Pongamia for mass production of biodiesel in the country.

Studies were cunducted on the variations within and between the naturally grown wild Pongamia plants in the North Karnataka region. Wild Pongamia trees were screened as candidate plus trees (CPTs) based on vegetative and productive traits by studying phenotypic and genotypic variations within and between the Pongamia ecotypes.

Highlights the variation studies on biochemical traits mainly on seed biochemical contents and protein were analyzed by SDS-PAGE and fatty acid profiling of seed oil by GCanalysis. However, the other biochemical traits like carbohydrate content, free amino acids content and oil properties like acid, saponification and iodine values were studied. The main objective was to study the polymorphism and variations in fatty acid compositions between Pongamia ecotypes.

Aimed to study the molecular diversity of ten selected candidate plus trees of Pongamia ecotypes selected from North Karnataka. The RAPD technique will generate data about the variations at genetic level which varies at regional level with a mean polymorphism percentage among the Pongamia ecotypes. ISSR marker analysis was based on small region variation in single locus which is not representative of total genome and the Pongamia ecotypes. The phylogenetic tree was constructed using TFPGA programme and analysis resulted low degree of polymorphism but significant difference were studied among the Pongamiaecotypes.

The study was conducted to standardize the protocol for *in vitro* multiplication of Pongamia. The use of plant regulatory hormones like 2, 4-D, BAP, Kin, AdS, TDZ, IAA, IBA, etc were helpful in developing techniques for *in vitro* multiplication of elite Pongamia. The establishment of protocol will be important for mass propagation and cultivation of elite

Pongamia clones. This will also help in genetic transformation studies in Pongamia.

Emphasize on genetic improvement of Pongamia using Diacyl glycerol acyltranferase (DGAT) enzyme which is exclusively committed to convert a diacyl glycerol to triacyl glycerol in fatty acid synthesis namely Kennedy pathway. Emphasis was also to synthesis cDNA using RNA isolated from developing seeds of Pongamia and partially amplify DGAT gene. The efforts were also made to establish an efficient protocol for infection of *Agrobacterium tumefaciens*harboring a binary vector pCAMBIA 2301 in Pongamia callus. This protocol will help in further studies on cloning and over expression studies of DGAT gene in Pongamia with improved TAG biosynthesis in seeds.

Highlights the comparison between the major biofuel plants grown in North Karnataka region especially Pongamia, Simarouba and Neem for various characters like yield and oil properties, biodiesel production, byproduct differences and biodiesel quality analysis.

The results from the studies have helped in drawing the major conclusions as follows

A total of 76 trees were collected and 30 wild Pongamia trees were screened as candidate plus trees (CPTs) based on vegetative traits like pod and seed characters, oil content, oil characters, etc followed by bioproductivity studies via plant height, collar diameter, canopy growth, number of branches per plant, number of leaves per branch, number of flowers per raceme, number of pods per bunch, seeds per branch, etc. The studies on phenotypic and genotypic variations, coefficient of variance, broad sense heritability and genetic advance have provided information about the qualities of plus tree and amount of characters which are passing to its off spring. The identification of CPT's of Pongamia ecotypes namely GRP8, 9, 13, 14, 28 and GRP29 are found superior in traits, namely 100 PW, 100 SW and OC. The correlation coefficient studies revealed interesting relationship between the pod and seed traits, these traits affect the germination of plant and these variations were observed in bioproductivity traits from germination toanthesis.

Variations in biochemical traits with reference to protein content, oil composition, oil characters are also important factors which were studied because they are affected by environmental conditions. Seed protein profiling based on the banding pattern studied by SDS-PAGE analysis showed difference in polypeptide bands at~190-150 kDa, 80-65 kDa and at 20-25 kDa which was helpful in further screening of CPTs of Pongamia. Fatty acid profiling was studied by GC analysis to study the variations in fatty acid composition among the Pongamia ecotypes. The variation in palmatic, stearic, oleic and linolenic acids were varying at a highest range, palmatic acid was among the major varying fatty acid ranging at 50-57 % which is identified as specific fatty acids responsible for production of biodiesel. Oil characters like acid, iodine and saponification value also play an important role in production of biodiesel as the amount of free fatty acid content and bonding pattern in fatty acids affects the quantity and quality ofbiodiesel.

PCR based molecular marker system via RAPD and ISSR markers also showed low polymorphism among the Pongamia ecotypes. A total of 27 RAPD and 13 ISSR primers were used for screening the genetic diversity among the CPTs of Pongamia, a total of 48 loci and 179 bands were produced by RAPD marker analysis and the polymorphism percentage was 22.35 %. Totally 88 loci and 141 bands were produced by ISSR marker analysis with a size ranging from 100 kb to 2.5 kb. The percentage of polymorphism ranged between 8-34 % with a mean value of polymorphism was 15.6 %. Phylogenetic tree were constructed based on RAPD and ISSR analysis showed good difference among the Pongamia ecotypes.

Standardization of protocol for induction of plantlet from Pongamia leaf explants was studied to produce callus using leaf and axillary bud explants at 4.52 µM 2,4-D in combination with 13.28 µM BAP. The explants responded to callus formation in less than a week and proliferation of shoots and multiple shoot were induction from callus which was studied at 13.28 µM BAP+ 9.1 µM TDZ which produces 9-13 shoots. The maximum rooting efficiency was observed in half strength MS media (½ MS) supplemented with 9.82 µM IBA, where 64 % and 59 % from leaf and axillary bud explants derived plants respectively. Well rooted plantlets were washed and transplanted into plastic pots containing sterilized soil and sand (1:1) for acclimatization, established in a greenhouse. The plant were gradually exposed to low humidity conditions and finally transferred for fieldtrails.

Genetic improvement of enzymes regulating the biosynthesis of triacyl glycerol (TAG) in seeds, Diacyl glycerol acyltranferase (DGAT) is the only enzyme exclusively committed to convert a diacyl glycerol to triacyl glycerol in fatty acid synthesis namely Kennedy pathway. During present work the synthesis of cDNA was acehived by isolating total RNA and partial amplification of DGAT gene of Pongamia, the primers were constructed using known nucleotide sequence of plants belonging to similar family. We were also successful to establish an efficient protocol for infection of *Agrobacterium tumefaciens*harboring a binary vector pCAMBIA 2301 in Pongamia callus. This was evident by GUS staining and also by PCR amplification of kanamycin (*npt II*) resistant gene amplified usinggenomic DNA from Agrobacterium infected callus. This offers a stable integration of transgenics using explants of Pongamia. This protocol will help in further studies on cloning and over expression studies of DGAT gene in Pongamia with improved TAG biosynthesis in seeds.

Comparison on yield, oil characters, biodiesel production and byproduct quantity and quality of biodiesel was studied between the other plants namely Simarouba and Neem which are also grown in North Karnataka region. Variation in results were observed in all the studied traits, the major traits of yield was observed in Pongamia which yield 5-6 tonnes of seeds per hectare, low oil density (0.91), free fatty acid content (2.24), viscosity of biodiesel (4.86), high quantity of biodiesel (895 ml/l of oil) and other studied traits makes Pongamia as the best plant species for production of biodiesel. Simaoruba and Neem has high oil density, free fatty acid content and high viscosity of biodiesel and less quantity of biodiesel production as compared to Pongamia; these parameters are important for production of high quality biodiesel. The lower the values of oil density, free acid content and viscosity of biodiesel, more stable is the biodiesel quality. Thus, these paramters makes Pongamia as a promising plant species among the other biodiesel plant species.

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